Depression vulnerability: Studying components of cognitive models

Kruijt, A.W.

chapter 4

The 5-HTTLPR polymorphism, early and recent life stress, and cognitive endophenotypes of depression

Kruijt, A.-W., Putman, P., & Van der Does, A. J. W. (2014)
Cognition & Emotion (28)
DOI:10.1080/02699931.2013.873018

Background: Studies associating interactions of 5-HTTLPR and life adversities with depression have yielded equivocal results. Studying endophenotypes may constitute a more powerful approach.
Aim: Assessing whether interactions of 5-HTTLPR with childhood emotional abuse (CEA) and recent negative life events (RNLE) affect possible cognitive endophenotypes of depression, namely attention allocation bias and the ability to recognize others' mind states.
Design: Association study in 215 young adults of North-West European descent.
Results: The ability to classify others' negative mind states was increased with increasing RNLE in carriers of low expressing 5-HTTLPR alleles. Carriers of two low expressing alleles also preferentially oriented attention towards negative information. Gene-environment interactions were not observed for attentional allocation bias. No effects involving CEA were observed.
Conclusion: Low expressing 5-HTTLPR alleles may confer increased risk for depression through enhanced recognition of negative facial expressions following recent negative life events.

The most studied polymorphism in relation to depression is the Serotonin Transporter Linked Polymorphic Region (5-HTTLPR), located in the promoter area of the gene encoding the serotonin transporter protein (SLC6A4; see www.HuGeNavigator.net; Yu, Gwinn, Clyne, Yesupriya, & Khoury). The 5-HTTLPR is a repeat polymorphism, resulting in short (S) and long alleles (L). The S allele is associated with reduced expression of the serotonin transporter protein, which regulates the reuptake of serotonin from the synaptic cleft. Within the L allele, a single nucleotide (guanine/adenosine) polymorphism exists (rs25531), resulting in L_g and L_a alleles. L_g alleles are regarded as functionally similar to the S allele (Hu et al., 2006). Carriers of one or two low expressing alleles (S or L_g) are found to be more sensitive to the effects of environmental adversity than L_A homozygotes. This interaction of 5-HTTLPR and environmental adversity on depression was first observed in a large cohort study (Caspi et al., 2003). Increasing levels of adversity (both childhood maltreatment before age 10 and recent negative life events between age 21-25) were associated with increasing probability of depression and suicidality at age 26 in S carriers, but not in L homozygotes (Caspi, et al., 2003). At that time the L_g/L_a distinction was not yet made. Despite a large number of studies attempting to replicate interaction effects of both early and recent life stress with 5-HTTLPR, the empirical evidence for these gene-environment interactions remains equivocal. Two meta-analyses found no evidence for a direct effect of 5-HTTLPR nor an interaction with recent life events (Risch et al., 2009) or recent and childhood stress (Munafó, Durrant, Lewis, & Flint, 2009) while a third meta-analysis supported interactions of 5-HTTLPR with both recent life events and childhood maltreatment (Karg, Burmeister, Shedden, & Sen, 2011).

These meta-analysis focused on depression as outcome measure. A possible partial explanation for the inconsistent results lies in the heterogeneity of the depression construct, which is likely influenced by a myriad of genetic and environmental factors. An upcoming alternative approach is to study associations with endophenotypes. Endophenotypes are hereditary biological or psychological processes or markers that precede or predispose to the pathology of interest (Gottesman & Gould, 2003; Lenzenweger, 2013). Because endophenotypes are more proximally related to the genotype than the disease itself (phenotype), associations between genotype and endophenotype may be more easily detected. Meta analyses on outcomes such as cortisol reactivity (Miller, Wankerl, Stalder, Kirschbaum, & Alexander, 2012), and developmental problems in children and adolescents (van Ijzendoorn, Belsky, & Bakermans-Kranenburg, 2012) support the existence of 5-HTTLPR by stress interactions.

Potential endophenotypes for depression include cognitive processing biases (Hasler, Drevets, Manji, & Charney, 2004). In the current study two types of processing bias are assessed as candidate endophenotypes: biased attentional allocation for emotional

information, and biases in recognition of emotional facial expression.

Biases in cognitive processing have been implicated in the aetiology of depression since the introduction of cognitive models (Beck, 1967). Among the biases associated with depression is preferential allocation of attention towards negative visual information. This bias is most often assessed with the dot probe task. Attentional bias for negative information has been observed in currently depressed (e.g. Fritzsche et al., 2009; Gotlib et al., 2004; Joormann & Gotlib, 2007), remitted depressed (e.g. Fritzsche, et al., 2009; Joormann & Gotlib, 2007), dysphoric (e.g. Bradley, Mogg, & Lee, 1997; Shane & Peterson, 2007), and at-risk (Joormann, Talbot, & Gotlib, 2007) samples. Several studies reported an additional, and dissociable, bias away from positive information (e.g. Fritzsche, et al., 2009; Joormann & Gotlib, 2007; Shane & Peterson, 2007).

Associations between 5-HTTLPR and attention allocation bias have also been reported. In the largest of these studies, healthy individuals homozygous for the L_a allele showed preferential orienting towards positive pictures and avoidance of negative pictures, which was not observed in carriers of S or L_g alleles (Fox, Ridgewell, & Ashwin, 2009). Other studies reported slightly different patterns of 5-HTTLPR effects, possibly due to the variation in genotype methods (e.g. assessment of L_a/L_g variants), outcome measure (dot probe task, Posner task, task features), and stimulus type (words, facial expressions, pictures). A meta-analysis concluded that individuals carrying two low expressing alleles show preferential orienting towards negative information (Pergamin-Hight, Bakermans-Kranenburg, van IJzendoorn, & Bar-Haim, 2012).

Only one study assessed possible gene-environment interactions involving 5HTTLPR and attention allocation bias in adults. Females carrying one or two S-alleles who reported childhood physical abuse selectively avoided angry facial expressions (Johnson, Gibb, & McGeary, 2010). However, only 13 participants in that study reported some degree of physical abuse, and the subsample of S-carriers must have been even smaller. Two other small studies in children reported evidence for attentional bias occurring as a function of 5HTTLPR and mothers' depression status (Gibb, Benas, Grassia, & McGeary, 2009), or mothers expressed criticism (Gibb et al., 2010).

Biases in the recognition of facial expressions of emotion are also associated with depression. Better recognition of negative expressions has been observed in remitted depressed samples (Anderson et al., 2011; Bhagwagar, Cowen, Goodwin, & Harmer, 2004), whereas currently depressed samples showed impaired recognition of negative facial expressions (Anderson, et al., 2011; Douglas & Porter, 2010). Facial emotion recognition is influenced by administration of selective serotonin reuptake inhibitors, which exert their action at the serotonin transporter (Anderson, et al., 2011; Bhagwagar, et al., 2004). Moreover, tryptophan depletion (a procedure to experimentally lower brain serotonin levels) reduces the ability to recognize fearful expressions in 5HTTLPR S carriers but not in L homozygotes (Marsh et al., 2006). A number of studies reported biased processing of negative facial expressions in children and adolescent samples as a function of 5-HTTLPR (e.g. Lopez-Duran, Kuhlman, George, & Kovacs, 2012; Székely et al., 2011) or an interaction between 5-HTTLPR and mothers' depression history (Jacobs et al., 2011).

One study assessed gene-environment interactions, between 5-HTTLPR and recent negative life events as well as childhood emotional abuse, on facial emotion recognition in adults (Antypa, Cerit, Kruijt, Verhoeven, & Van der Does, 2011). Carriers of a low expressing allele who experienced recent negative life events recognized angry and sad facial expressions better. Furthermore, L_A homozygotes reporting childhood emotional abuse showed impaired recognition of angry facial expressions.

The current study is intended as a conceptual replication of the findings by Antypa and colleagues (2011), and a replication and extension of the findings by Fox and colleagues (2009). We extended the design of the latter study by also assessing gene-environment interactions, while using a very similar measure of attention allocation bias. For the conceptual replication of the findings by Antypa and colleagues (2011), a different measure of emotion recognition was used. The Reading the Mind in the Eyes Test (RMET; Baron-Cohen, Wheelwright, Hill, Raste, & Plumb, 2001) assesses the ability to recognize relatively complex emotional states (e.g., grateful, concerned), rather than basic emotional expressions (e.g., happy, fearful). Similar to studies assessing recognition of basic facial emotional expressions, RMET studies yielded a pattern of increased mind state recognition in dysphoric students and remitted depressed participants (Harkness, Jacobson, Duong, & Sabbagh, 2010; Harkness, Sabbagh, Jacobson, Chowdrey, & Chen, 2005), and reduced recognition in currently depressed patients (Lee, Harkness, Sabbagh, & Jacobson, 2005), although this was not always found (Wolkenstein, Schönenberg, Schirm, & Hautzinger, 2011).

Gene-environment interaction effects were assessed using the same environmental stress measures as in the study by Antypa and colleagues (2011). Self-reported negative life events that occurred during the six months preceding the test were used as an index of recent negative life events. Self-reported childhood emotional abuse was used to index childhood adversity.

We expected carriers of at least one low expressing alleles (S/L_g) to show attentional bias towards negative information and away from positive information, whereas L_a homozygotes were expected to show a relative bias towards positive and away from negative information. We also expected a moderation effect of exposure to early or recent life events, reflecting less stress sensitivity in L_a homozygotes. Considering that our sample was not currently depressed, we expected that carriers of low expressing alleles (S or L_g) would perform better at recognizing others' mind states if they had been exposed to early or recent life stress.

Methods

Participants

Participants were recruited through posters and flyers. Participants were between 17 and 35 years old, were not currently depressed, had normal or corrected to normal vision, and were of middle and northern European descent (all four grandparents born in a region spanning from France to Austria, up to Scandinavia). Data were obtained between February and October 2011.

Measures

Attentional bias

Preferential orienting of attention was assessed with a dot probe task (MacLeod, Mathews, & Tata, 1986). Each trial started with a fixation cross shown in the middle of the display for 500 ms, followed by two stimulus pictures in horizontal arrangement for 500 ms. Upon offset of the stimuli, a small figure (the probe) appeared in the middle of the location previously occupied by either stimulus picture. Participants were instructed to respond to the identity of the probe as fast as possible by pressing either one of two buttons on a mouse that was attached to the desk in front of the participants. The correspondence between mouse buttons (left/right) and probe identities (square or diamond shape) was counterbalanced across participants. Stimulus pictures had a positive, negative or neutral valence and were selected from the International Affective Picture Set based on valence and arousal ratings (IAPS; Lang, Bradley, & Cuthbert, 1999). Negative pictures depicted depression related rather than threatening scenes. See supplementary material for a more detailed description of the dot probe task features, randomizations, and stimulus pictures.

Bias indices were calculated by subtracting response times on trials wherein the probe appeared at the location previously taken by an emotional stimulus (congruent trials) from response times on incongruent trials (MacLeod, et al., 1986). A positive value indicates preferential orienting of attention towards the emotional stimulus.

Reading mind states

The Reading the Mind in the Eyes Test (RMET) was adapted for computerized assessment. Participants received written instructions, followed by a test trial and 36 experimental trials. Within each trial, a stimulus picture was shown at the centre of the display, with four response options at its corners, until participants responded by mouse clicking. The positions of the four answer options were randomized. Our participants were students who were native Dutch and competent at English, therefore both the original English words as well as the Dutch translations were presented side by side. As in the original version, a glossary was provided for reference during the task, listing the English and the Dutch words, their meaning, and an example sentence. No time limit was set for answering, but participants where instructed not to think too long before answering. The 36 trials of the RMET included 8 positive trials, 12 negative trials and 16 neutral or 'other' trials, according to the valence of the correct response (Harkness, et al., 2005, pp. table 1, p 1007.).

Childhood Trauma
The Childhood Trauma Questionnaire – Short Form (CTQ-SF; Bernstein et al., 2003) is a 28 item questionnaire assessing five categories of childhood trauma: emotional abuse, physical abuse, sexual abuse, emotional neglect, and physical neglect. Items are rated on a 5-point Likert scale anchored from (1) never true to (5) very often true. The emotional abuse scale consists of five items assessing whether the respondent felt loved, felt looked out for, was made to feel important, and whether the respondent felt that family was close and a source of strength (Bernstein, et al., 2003).

Recent Negative Life Events
The number of negative life events that occurred during the six months preceding the test was assessed using the List of Threatening Experiences – Questionnaire (LTE-Q; Brugha, Bebbington, Tennant, & Hurry, 1985; Brugha & Cragg, 1990). This questionnaire assesses the occurrence of twelve negative life events such as illness or injuries to the self or close friends and relatives, loss of friends, relatives or partners, loss of job or housing, and being victimized by theft or assault. The LTE-Q is often used in studies assessing possible relationships between recent negative life events, 5-HTTLPR, and depression (Risch, et al., 2009, p. 2464). The derived information is in high agreement with information obtained from interviewing participants or their relatives, and the test-retest reliability is high (Brugha & Cragg, 1990).

Depression
Current and past incidence of major depressive episodes was assessed with the Major Depression Questionnaire (MDQ; Van der Does, Barnhofer, & Williams, 2003). This self-report list assesses the presence of all diagnostic criteria for a major depressive episode. This is done twice, once for a period of two weeks during the past month, and once for any period of two weeks during lifetime. Responses were scored according to DSM-IV criteria for major depressive disorder (American Psychiatric Association, 2000). Participants reporting sufficient symptoms to suggest that a diagnosis of major depressive episode applied to any period of two weeks during the past month were excluded. This should be considered a strict criterion. Comparison of 39 diagnoses derived from the MDQ and the SCID interview (Spitzer, Williams, Gibbon, & First, 1994), suggested that the MDQ has a sensitivity of 100%, yet a specificity of 75% (Williams, Van der Does, Barnhofer, Crane, & Segal, 2008).

Genotyping
Saliva samples were collected in Oragene Self-Collection Kits – DISC format (DNA Genotek Inc, Ottawa, Ontario, Canada). See supplementary material for a description of the PCR and genotyping procedures.

Procedure
Participants were scheduled for 1.5 hours appointments. Upon arrival at the laboratory, participants received written and verbal information on the purpose and procedure of the study and were given the opportunity to ask questions before signing informed consent. The RMET and the dot-probe were the first of four computerized behavioural tasks, followed by computerized administration of questionnaires. At the end of the procedure

participants provided a saliva sample for genotyping. Participants rinsed their mouth during a short break approximately 30 minutes before providing the saliva sample. Finally, participants were debriefed and thanked. Participants received a small compensation for participating. The medical ethics committee of Leiden University Medical Center approved the study protocol.

Analyses

Moderated regression analyses were used to assess interactions of 5-HTTLPR and environmental stress. Analyses started with assessment of bivariate relations, i.e. correlations, between the outcome variables (attentional bias positive, attentional bias negative, RMET total, RMET positive items, and RMET negative items), and the predictors (5,HTTPLR, CEA, RNLE, sex and MDQ status). If sex or MDQ status had been identified as a possible covarying variable, i.e. found to be associated with an outcome variable, it would be included in the subsequent moderated regression analyses. If no possible confounders were identified, the simplest possible model was used to assess significance of the gene-environment interaction term. The simplest model includes three terms: 5-HTTLPR, CEA or RNLE, and the corresponding gene*environment interaction. Significant gene-environment interactions were further explored using simple slopes analyses (Aiken & West, 1991).

The CEA predictor represents the CTQ-CEA score, which has a minimum of 5. Scores of 5, 6, 7, etc. points were recoded as 0, 1, 2, etc. RNLE were coded as 0, 1, 2, etc. for or 0, 1, 2, etc. events. Allelic variants were coded as 0 (SS, SL_g, L_gL_g), 1 (SL_a L_gL_a), or 2 (L_aL_a), representing the number of, high expressing, L_a alleles (Caspi, et al., 2003). Sex was dummy coded (0=male, 1=female), as was self-reported history of major depression (MDQ status, 0=no, 1=yes). For the moderated regression analyses, variables were mean centred (Aiken & West, 1991).

Results

Participants

Data were obtained for 238 participants. Twenty-three participants reporting symptoms indicative of a major depressive episode within the month preceding testing were excluded. Analyses were based on 215 participants. Due to a procedural error, one participant took the dot probe task twice, while the RMET was not administered. Data obtained in the first dot probe assessment were used and this participant was excluded from the RMET analyses.

Gene data

Amongst the 215 participants, 47 were carriers of two low expressing alleles (low expression group: 40 SS, 6 SL_g, 1 L_gL_g), 98 carried one low and one high expressing allele (medium expression group: 89 SL_a, 9 L_gL_a), and 69 participants were homozygous for the L_a allele (high expression group: 69 L_aL_a). The observed distribution of genotypes (S and L_g alleles collated) was in Hardy-Weinberg equilibrium: $\chi^2_{(1,\, n=215)} = 1.36$, $p = .24$. This was also the case for the distribution of the three allelic variants ($S/L_g/L_g$): $\chi^2_{(3,\, n=215)} = 3.00$, $p = .39$). For the remainder of this paper, the terms low, medium and high expression will be used

to indicate 5-HTTLPR groups.

Table 4.1. *demographic information per 5-HTTLPR group.*

5-HTTLPR:	low (*n*= 48)		medium (*n*= 98)		high (*n*= 69)		
	n	%	*n*	%	*n*	%	*p*
female	42	87.5	86	87.8	57	82.6	.606
MDQ status	17	35.4	34	34.7	27	39.1	.833
	M	sd	M	sd	M	sd	
age	20.0	2.2	20.0	2.9	20.1	2.9	.946
CEA	6.8	2.6	7.0	2.3	7.4	3.3	.490
RNLE	1.1	1.0	1.2	1.2	1.2	1.2	.816

For sex and self-reported history of depression (MDQ status), the reported p value is associated with X^2 tests, all other p values are associated with one-way ANOVA F-tests. 5-HTTLPR low = SS, SL_g, L_gL_g, medium = SL_a, L_gL_a, and high = L_aL_a. MDQ status = self reported symptoms indicate past major depressive episode(s), CEA = Childhood Emotional Abuse, RNLE = Recent Negative Life Events. Based on non-centred variables.

Demographics

Table 4.1 lists the demographic and environment measures per 5-HTTLPR group. Chi-square tests and one-way ANOVAs indicated no differences between 5-HTTLPR groups.

Data preparation

Attentional bias

Error trials were removed. On average, participants made 4% errors. Three participants were identified as outliers with respect to error rate (threshold = M+3SD = 14%) and were excluded from further analysis. Trials with response times below 200 or above 2000 ms were discarded (0.2% of remaining trials). The remaining data showed positive skew and kurtosis, therefore median instead of mean values were used to derive bias indices (MacLeod, Rutherford, Campbell, Ebsworthy, & Holker, 2002). Bias indices for positive-neutral and negative-neutral trials were calculated by subtracting the median response times for congruent trials from the median response times for incongruent trials.

Reading mind states

Percentage correct scores were calculated for the total number (36) of RMET trials, and separately for the eight positive and the twelve negative trials (Harkness et al., 2005; table 1, p 1007).

Data inspection

Most variables were approximately normally distributed, only the CEA distribution showed positive skew (2.08) and kurtosis (5.0). Because more extreme data points may disproportionally influence analyses outcomes, scatterplots were inspected to assess possible influences of the distribution on the bivariate analyses. For the moderated regression analyses, Cook's distances and the distribution of the residuals were inspected

to identify influential data points. If such cases were identified, analyses were repeated excluding these cases. Although the RMET positive item score was approximately normally distributed, three possible outliers were identified, each with a 25% correct score. These were initially retained, but analyses on this variable were also repeated excluding these cases.

Table 4.2. *bivariate associations*

		1	2	3	4	5	6	7	8	9
1	DP BI pos.	-								
2	DP BI neg.	.01	-							
3	RMET total	.07	.00	-						
4	RMET pos.	.04	-.02	.40***	-					
5	RMET neg.	-.02	.04	.70***	-.05	-				
6	sex	-.04	-.11	.09	.04	.11	-			
7	MDQ status	.11	-.09	.06	.01	.11	.00	-		
8	CEA	-.01	.01	.06	-.02	.10	-.01	.34***	-	
9	RNLE	-.04	.07	-.02	-.07	.07	.00	.17*	.20**	-
10	5-HTTLPR	-.02	-.14*	.03	.08	-.02	-.06	.03	.08	.02

*Pearson's correlations; * < .05, ** < .01, *** < .001.*
Pos. = positive, neg. = negative, DP BI = dot probe Bias Index, RMET = Reading the Mind in the Eyes Task, CEA = Childhood Emotional Abuse, RNLE = Recent Negative Life Events, MDQ status = self reported symptoms indicate past major depressive episode(s)

Bivariate analyses

Correlations between all variables are shown in table 4.2. As would be expected, RMET total score was highly correlated with the scores for RMET negative items (.70) and positive items (.40). All other correlations were small to moderate in size, and most were non-significant. Attention allocation bias for positive and negative stimuli were not associated, nor were these associated with RMET assessed bias. Self-reported history of depression (MDQ status) and sex were not related to any of the outcome variables (attention allocation or mind state recognition), ruling them out as possible covarying variables. CEA and RNLE were significantly associated (r =.20, p = .004), and both were associated to MDQ status (CEA: r = .40, p < .001; RNLE: r = .18, p = .011). These associations may reflect either a causal role of CEA and or RNLE in the aetiology or depression, or biased reporting of CEA and RNLE by participants with a history of depression. No correlations were observed between 5-HTTLPR groups and the life event variables (RNLE: r = -.001; p = .99, CEA: r = .08; p = .24), ruling out gene-environment correlations.

In line with our first hypothesis, a weak association between 5-HTTLPR and attention allocation bias for negative items was observed (r = -.14, p = .049). T-tests yielded a significant difference in allocation bias index for negative information between the low and high expression homozygous groups ($t_{(113)}$ = 2.03, p=.044), but not between the heterozygous and either of the homozygous groups (both p > .05), also see figure 4.1. None of the observed bias indices differed from zero (all p > .05). Thus, carriers of two low expressing alleles preferentially oriented attention towards negative information, whereas L_a homozygotes showed a relative tendency to avoid negative information. The hypothesized association between 5-HTTLPR and attention allocation bias for positive stimuli was not observed.

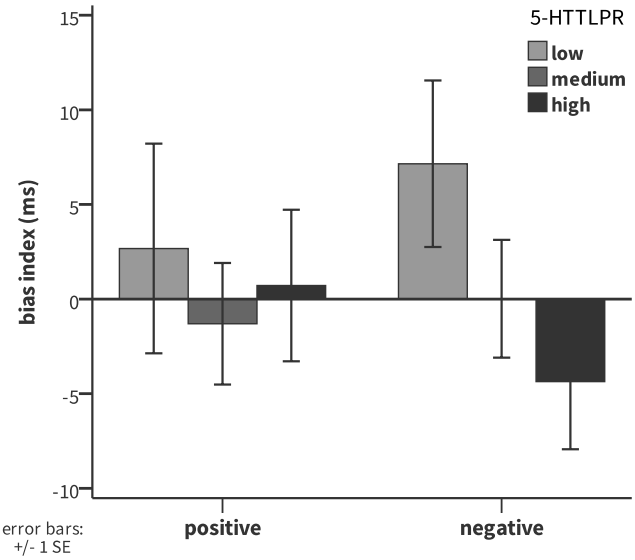

Figure 4.1. attentional bias for positive and negative information by 5-HTTLPR.
low = SS, SL_g, L_gL_g, medium = SL_a, L_gL_a, high = L_aL_a
* t(113) = 2.03, p=.044

All bivariate associations pertaining to the skewed variable CEA were non-significant. Inspection of scatterplots did not suggest that the distribution of CEA influenced associations in any such way that possible significant correlations were obscured. Repeating the bivariate analyses excluding the three, previously identified, possible outliers on RMET positive item score did not meaningfully change outcomes.

Moderated regression analyses:
The moderated regression analyses are presented in full in tables 4.s2a and 4.s2b of the supplemental information.

Attentional bias
Moderated regression analyses yielded no significant interaction effects for 5-HTTLPR by RNLE or for 5-HTTLPR by CEA on attentional bias indices for positive or negative information (see table 4.3).

Reading mind states
A significant interaction of 5-HTTLPR and RNLE was found for RMET negative item scores (b= -2.54, se =1.21, $t_{(210)}$ = 2.10, p =.037; model r^2 = .026). The direction of this interaction was such that carriers of two low expressing alleles (S or L_G) showed increased recognition of negative mind states with increased exposed to negative life events (figure 4.2a). Simple

slope analyses were significant for the low expression group (t(209) = 2.33, *p*= .021), but not for the intermediate (*t*(209) = 1.45, *p* =.148), or high expression groups (*t*(209) = -.97, *p* = .333; figure 4.2b). No other gene-environment interactions were found significant, see table 4.3.

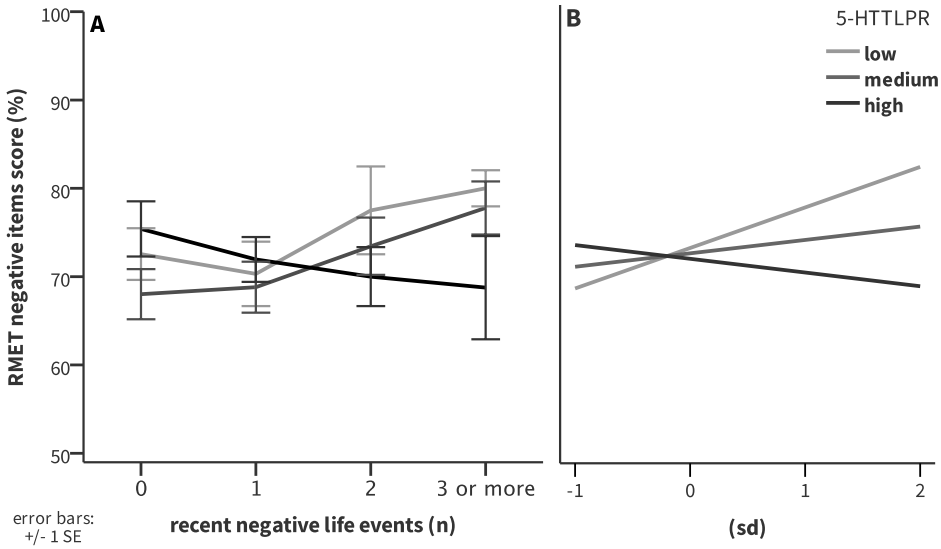

Figure 4.2. 5-HTTLPR by RNLE interaction on RMET negative item score
A) RMET negative item score by RNLE and genotype B) simple slopes plot
5-HTTLPR: low = SS, SL_g, L_gL_g, medium = SL_a, L_gL_a, high = L_aL_a

Influential data points

For all analyses, values of Cook's distance were lower than 1 (maximum values per analysis ranged from .05 to .49). The analyses for RMET positive item scores were repeated without the previously identified possible extreme outliers. All analyses were repeated with standardized residuals < -3 and > 3 removed. Per outcome measure 0 to 4 cases were excluded to achieve that all residuals fell in the -3 to 3 range. Re-analyses did not change the initial outcomes.

Table 4.3. *characteristics of the observed gene-environment interaction effects for each outcome measure and operationalization of adversities (CEA/RNLE).*

	5-HTTLPR * CEA				5-HTTLPR * RNLE			
	B	*SE*	*t*	*p*	*B*	*SE*	*t*	*p*
DP BI pos.	-.90	1.09	-.83	.409	1.70	2.82	.60	.548
DP BI neg.	.64	.98	.65	.518	1.70	2.53	.67	.502
RMET total	.17	.29	.59	.554	-.55	.76	-.72	.472
RMET pos.	-.34	.52	-.65	.519	-.89	1.34	-.66	.508
RMET neg.	-.22	.47	.47	.643	-2.54	1.21	-2.10	.037

*Pos. = positive, neg. = negative, DP BI = dot probe Bias Index, RMET = Reading the Mind in the Eyes Task, CEA = Childhood Emotional Abuse, RNLE = Recent Negative Life Events. Interaction effects assessed in moderated regression models with three predictors: 5-HTTLPR, adversity (CEA/RNLE), and interaction 5-HTTLPR*adversity.*

Additional analyses:

We adopted the statistical method used in he initial paper (Caspi, et al., 2003) and most papers assessing 5-HTTLPR*stress interactions, including one meta-analysis (Risch, et al., 2009). In these regression analyses, 5-HTTLPR is handled as a linear variable, implicating a linearly additive genetic model. A less often used, alternative, approach is to contrast all carriers of a low expressing allele (SS or L_g) with the L_a-homozygotes. A dichotomized representation of 5-HTTLPR complies better with assumptions of regression analysis, but ignores possible variation associated with carrying one versus two low expressing alleles. Analyses were repeated with 5-HTTLPR recoded, such that all S or L_g carriers were coded as 0, and L_a homozygotes as 1 (see tables 4.s3a and 4.sb in the supplemental information). For most analyses outcomes did not chance meaningfully (see supplementary material). The interaction effect of 5-HTTLPR and RNLE was again found significant for RMET negative item score (b = -4.44, se = 1.78, $t_{(210)}$ = -2.49, p = .013, model r^2 = .034). In line with the main analyses, subsequent simple slope analysis was significant for S or L_g-carriers ($t_{(210)}$ = 2.27, p = .024), but not for L_a homozygotes ($t_{(210)}$ = -1.44, p= .151). The bivariate association between allelic variant and attentional bias for negative information was not found when contrasting S/L_G-carriers and L_A homozygotes (r= -.103, p= .134). Moreover, most papers to date assessing main effects (but not gene-environment interaction) of 5-HTTLPR on attentional bias allocation utilized factorial ANOVA analysis, treating 5-HTTLPR as a nominal variable without implicating a genetic model. When no linear model was assumed in ANOVA, the effect of genotype on attentional allocation bias for negative information was not significant: $F_{(2,209)=}$2.010, p=.137. Assessment of the linear contrast in ANOVA yielded a result similar to the bivariate linear analysis: $F_{(1,209)=}$ 3.912, p = .049 (weighted contrast).

Discussion

An interaction effect between 5-HTTLPR and recent negative life events influencing recognition of negative mind states was observed. A weak association was found for RNLE and attentional bias for negative information. No gene*environment interactions were observed for attention allocation bias, nor were any effects involving early life stress (CEA).

The observed interaction effect is a conceptual replication of previously reported improved recognition of angry and sad facial expressions following negative life events in carriers of two low expressing alleles (Antypa, et al., 2011). Effects similar to the previous finding of impaired recognition of angry expressions by L_A homozygotes exposed to CEA (Antypa, et al., 2011) were not observed. This may because the RMET does not assess the recognition of discrete basic emotional expressions (e.g., anger, sadness, disgust), but rather more complex negative affects. Alternatively, the non-replication of any CEA effects may be ascribed to low CEA incidence, as discussed below.

Hypothesized gene-environment interactions affecting attentional allocation bias were not found. The observed main effect of 5-HTTLPR on attentional allocation bias for negative information is a replication of previous findings in smaller samples (meta-analysis: Pergamin-Hight, et al., 2012). However, this effect was only just significant (p = .049) and dependent on the statistical method: it was only found if a linearly additive genetic model was implied in the statistical model, and it was not found when carriers of either one or two low expressing alleles (S or L_G) were collated. A previously reported main effect of 5-HTTLPR on attentional allocation bias for positive information was not found, although a dot probe task with very similar stimuli (IAPS pictures; Lang, et al., 1999) and the same exposure duration (500 ms) was used (Fox, et al., 2009).

With sample sizes in previous studies ranging from n= 27 to n = 106 (Beevers, Gibb, McGeary, & Miller, 2007; Fox, et al., 2009; Johnson, et al., 2010; Kwang, Wells, McGeary, Swann Jr, & Beevers, 2010), the sample in the current study (n=215) was twice as large as the sample is largest study to date assessing 5-HTTLR effects on attention allocation bias in adults. Our sample was slightly smaller than the only other study assessing 5-HTTLPR effects on facial emotion recognition (n = 245; Antypa, et al., 2011).

Given a sample size of 215, effect sizes of f^2 = .036 and larger can be detected with at least 80% power in linear regression models with three predictors. In the context of behavioural science, the effect size measure f^2 was recommended to be interpreted as small at a value of .02, medium at .15, and large at .35 (Cohen, 1992). The observed effect size of the significant 5-HTTLPR RNLE interaction model was f^2 = .026, associated with an estimated achieved power of .65. Therefore, still larger studies are needed to confirm these findings.

Associations between a genotype and an endophenotype, e.g. cognitive bias as assessed in this study, may be relatively easy to detect compared to associations between a genotype and a disease of interest, because a more proximal relation is likely influenced by fewer other factors. However, the endophenotype, i.e. the presence of bias, does not necessarily

result in depression incidence. Thus, the currently reported interaction suggests that carrying S or L_g alleles may confer an increased risk for developing depression in response to negative life events, through increased recognition of negative facial emotion following negative life events. Meta-analyses and longitudinal studies may confirm whether the relationship between 5-HTTLPR, stress, and depression is indeed mediated by biased processing of emotional information.

Additional studies are also needed to extend establish that biased information processing indeed qualifies as an endophenotype. Five criteria have been suggested: that the endophenotype is associated with the phenotype in the population, is heritable, is state-independent, co-segregates with the phenotype within families, and is found in non-affected members of affected families at a higher rate than in the general population (Gottesman & Gould, 2003, p. 639). Several studies on biased information processing provided initial evidence for several of these criteria, yet we are not aware of any studies explicitly assessing these endophenotype criteria for biased attentional allocation or facial emotion recognition. Given the current results, we suggest that such future studies focus on biased facial emotion recognition.

It has been suggested that the assessment of environmental adversity with self-report measures is inferior to interview-based assessment. Stress-moderated 5-HTTLPR effects were more often reported in studies utilizing interview-based measures (Uher & McGuffin, 2007, 2010). In the present study, we observed an interaction effect involving self-reported RNLE. We would like to forward the consideration that interviews are more typically used in smaller sized studies, which could also explain why an association between interview assessment and positive results has emerged. Additionally, the idea that the emotional impact of negative life events is more accurately assessed in an interview also suggests that a confounding may occur between this measure and depression-related outcomes. Nonetheless, the use of retrospective self-report measures for environmental adversity (RNLE and CEA) should be considered a limitation of the present study, as these are vulnerable to recollection bias. The extent to which such bias occurs may also differ between gene variants. In our sample, zero correlation between allele variants and the environmental stress variables was observed. Suggesting both that 5-HTTLPR did not moderate possible self-report bias, as well as absence of gene-environment correlation. Nonetheless, future studies should consider assessing more objective as well as prospective assessment of negative and positive environmental factors.

Another limitation of indexing life stress would remain, namely their low incidence. In the current sample, 6-month incidence of RNLE ranged from 0 to 6 negative events, with a mean of 1.2 events. Sixty-eight per cent of the sample reported at least one negative event, and 30% reported more than one. A longer indexation period would have resulted in higher incidence, yet this could have been at the cost of specificity of RNLE impact.

A limitation of our study is the low CEA incidence, which may explain the absence of the hypothesized CEA effects. Sixty-four per cent of our participants reported some amount of emotional abuse (score 6 or higher). This is similar to the average for both clinical and community samples (Baker & Maiorino, 2010, p.743 table 2). The average level of CEA

reported in the current sample is only slightly, likely not significantly, lower compared to previous studies wherein 5-HTTLPR CEA interactions were observed. However, the authors of the CTQ-SF proposed that a cut-off score of 9 represents at least low emotional abuse (Bernstein & Fink, 1998). A score higher than nine was observed in only 43 of our participants (20%), which is low compared to the average proportion (42%) in community samples (Baker & Maiorino, 2010, p.743 table 2).

Alternatively, one may argue that our finding of no CEA interactions effects fits with cognitive models of depression, considering that our sample was never or not currently depressed. Cognitive models state that negative early experiences (e.g. CEA) may shape a tendency for dysfunctional cognitive processing. However, in adult life dysfunctional cognitive processing is expected to remain latent unless activated following adverse events. Thus, while interaction of 5-HTTLPR and CEA was repeatedly observed in studies assessing effects on depression prevalence, processing biases as a result of 5HTTLPR interacting with CEA could arguably be expected to be 'inactive' in non-depressed individuals when not triggered by RNLE. Following the initial report of 5-HTTLPR interacting with both early and adult life adversities in predicting depression (Caspi, et al., 2003), the forthcoming literature often did not distinguish between these two interactions. A recommendation for future studies assessing 5-HTTLPR by stress interactions on cognitive endophenotypes is to consider theoretical distinctions between childhood and recent life stress.

The current findings suggest that 5-HTTLPR may differentially affect attention allocation bias and reading others' mind states. Speculatively, pending replication, this pattern may be explained by attentional bias reflecting relative automatic processing with less higher-order cognitive involvement than mind state recognition. Future studies could compare implicit and explicit measures of cognitive processing. A pattern of stress moderation on an explicit but not an implicit measure of depression related cognition was observed in a study informed by a dual processing theory of depression (Haeffel et al., 2007). In addition, an interaction of 5-HTTLPR and CEA has been reported for an explicit measure of cognitive reactivity to sad mood (Antypa & Van der Does, 2010).

To summarize, we report tentative evidence of a direct effect of 5-HTTLPR on attention allocation bias, such that individuals carrying two low expressing alleles (S or L_g) showed a relative bias towards negative visual information, compared to those homozygous for the L_a allele. Importantly, this effect was dependent on the implication of a linear model in statistical analysis. No main effect on allocation bias for positive information and no interaction effects of 5-HTTLPR and CEA or RNLE on attention allocation bias were observed. For the ability to recognize other's mind states, an interaction effect of 5-HTTLPR and RNLE was found. This finding suggests that increased risk for depression in carriers of low expressing 5-HTTLPR alleles could be due to enhanced recognition of negative facial expressions following negative life events. Hypothesized interactions between 5-HTTLPR and CEA were not observed. We argue that future studies testing the endophenotype approach may distinct between implicit and explicit measures and, on theoretical grounds, focus on interactions of 5-HTTLPR and RNLE.

Acknowledgements
Analyses of different outcome measures and another polymorphism acquired in the same sample have been reported elsewhere (Drost, Spinhoven, Kruijt, & Van der Does, 2013; Verhoeven et al., 2012). The authors would like to thank Anne Junggeburt, Fabrizio Derubeis, Jessica van Leeuwen, Lili Chu, Ludo Seip, Nadin Mousa, Sebastian Potthoff, Stefan van Liempt, and Stephanie Harmsen for assisting with the data collection for this study. This research was funded by an N.W.O. Vici grant (# 453-06-005) to A.J.W.V.D.D.; P.P. is supported by an N.W.O. VIDI grant (#452-12-003).

Supplemental information

Dot probe task additional details

Stimulus pictures and selection
Stimulus pictures were positive, negative, and neutral pictures selected from the International Affective Picture Set (IAPS; Lang, et al., 1999). Picture selection was based on ratings of valence and arousal, and subsequent selection by AWK. The final selection was discussed and agreed upon by all three authors.

The initial selection of candidate pictures was based on mean valence and arousal ratings provided with the IAPS (based on 9-point Likert scales). Boundary scores used for the initial selection were: valence > 6 & arousal > 5 for positive stimuli; valence < 4 & arousal >5 for negative stimuli; valence 4.5 – 5.5 & arousal < 4.5 for neutral stimuli. A subsequent selection was made ensuring that negative pictures represented depressotypic as much as possible, that pictures where not likely to be perceived much different by our Dutch participants compared to the American raters, and that neutral pictures depicted neutral rather than ambiguous scenes (e.g. picture #4233, for which the ratings fall within our predefined neutral range, depicts a street prostitute, which may not have been recognized by all raters). Stimuli depicting people or human-related scenes (e.g. a cemetery) were preferentially selected. A total of twenty positive, twenty negative and forty neutral pictures were selected.

The selection procedure ensured that valence and arousal ratings differed significantly over the three categories (one way ANOVA's, both p < .001). Paired sample t-tests for each combination of valences showed that positive and negative pictures did not differ in their arousal ratings (t(38)= .03, p =.978), whereas arousal ratings for neutral stimuli differed from both positive and negative stimuli (t(58) = 17.42, p = <.001 and t(58) = 17.40, p = <.001). Valence ratings differed significantly between neutral and positive (t(58) = 23.25, p = <.001), neutral and negative (t(58) = -35.27, p = <.001), and positive and negative stimuli (t(38) = 36.29, p = <.001), also see figure 4.s1.

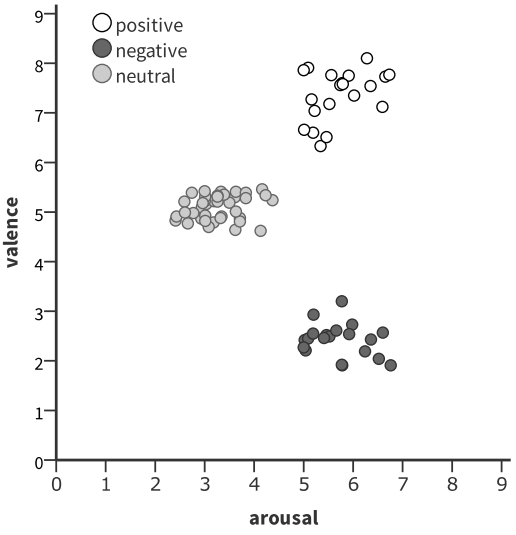

Figure 4.s1. valence and arousal ratings for the selected stimuli
(based on Lang, et al., 1999).

Selected pictures:
positive:
1722, 2058, 2071, 4617, 4626, 4640, 5470, 5622, 5628, 7502, 8033, 8080, 8180, 8190, 8200, 8370, 8380, 8420, 8496, 23521

negative:
2688, 2703, 2710, 2799, 2900, 3030, 3180, 3220, 3550, 6570, 9050, 9120, 9250, 9419, 9421, 9423, 9435, 9520, 9530, 9921

neutral:
1675, 2038, 2102, 2190, 2191, 2200, 2210, 2305, 2357, 2381, 2393, 2396, 2397, 2410, 2441, 2445, 2480, 2495, 2514, 2575, 2595, 2840, 2850, 2870, 2880, 5395, 5471, 5500, 5731, 5740, 7009, 7036, 7037, 7038, 7041, 7242, 7493, 7500, 9070, 27451

Contrary to Fox and colleagues (Fox, et al., 2009; personal communication), we did not create subsets based on arousal ratings (< 4.5 versus > 6) within the valence types. Thus, in our study any observed emotional versus neutral differences (e.g. bias) should be ascribed to a combination of valence and arousal differences. Any observed differences in positive versus negative bias can be ascribed to valence, because these categories do not differ in arousal ratings.

Pictures were converted to gray scale to prevent attention being drawn by either picture within a pair due to color rather than valence differences, and the pictures were resized to 453 by 340 pixels. Presented on the display, the horizontal distance between the pictures was 315 pixels, while participants were seated at approximately 60 cm distance from the display. Therefore, each stimulus subtended approximately 11.3° of visual angle in the horizontal plane and the probe approximately 0.4°. The distance between two stimuli subtended approximately 8°, and the distance between the two possible probe positions about 19.6° of visual angle in the horizontal plane. The probe was a 15*15 pixels black square that was shown either upright (square) or tilted 45° (diamond). These probes are identical in shape and size, and only differ in their orientation.

procedure details:
Within a single administration of the task (320 trials), each of the 80 stimulus pictures was used 8 times. One session consisted of 80 positive-neutral trials, 80 negative-neutral trial, 80 neutral-neutral trials and 80 same valence trials (40 positive, 40 negative). Within trials of each category, the stimulus pictures, the position of the emotional stimulus, the position of the probe (location previously taken by the emotional or by the neutral stimulus) and the identity of the probe were counterbalanced and administered in random order. A short self-paced break was given following every 30th trial: a message appeared on the display, advising the participant to take a moment of rest before continuing the task by means of a button click.

PCR and genotype procedure:
Saliva samples were collected in Oragene Self-Collection Kits – DISC format (DNA Genotek Inc, Ottawa, Ontario, Canada). Approximately 10 ml of saliva was kept in 2ml lysis buffer

(100 mmol/L NaCl, 10 mmol/L EDTA, 10 mmol/L Tris pH 8, 0.1 mg/mL proteinase K, and 0.5% w/v sodium dodecyl sulfate) until further processing.
Triplex polymerase chain reaction amplification (PCR) was used to amplify the region of interest from the SLC6A4 gene, with the following primers: a FAM-labeled primer HTTLPR-FWFAM 5'-TCCTCCGCTTTGGCGCCTCTTCC-3', and a reverse primer HTTLPR-RV 5'-TGGGGGTTGCAGGGGAGATCCTG-3'. Typical PCR reactions contained between 10 and 100 ng genomic DNA template, and 10 pmol of forward and reverse primer. PCR was carried out in the presence of 5% DMSO with 0.5 U of BioThermAB polymerase (GeneCraft, Munster, Germany) in a total volume of 30 µl. The cycling conditions were as follows: an initial denaturation step of 5 min at 95 °C, followed by 40 cycles of 30 seconds at 96 °C, 30 seconds at 61 °C, 60 seconds at 72 °C and a final extension step of 10 minutes at 72 °C. One µl PCR product was mixed with LIZ-500 size standard and formamide and run on an AB 3100 genetic analyzer setup for genotyping with 36 cm capillaries. Results were analysed using GeneMarker software (Softgenetics).

Simple slopes analyses:

Regression model:

$$y = \beta_0 + \beta_{5\text{-HTTLPR}} * x + \beta_{RNLE} * x + \beta_{5\text{-HTTLPR*RNLE}} * xz$$

can be rewritten as:

$$y = (\beta_{RNLE} + \beta_{5\text{-HTTLPR*RNLE}} * x) * z + (\beta_0 + \beta_{5\text{-HTTLPR}} * x)$$

The first part of the above formula is the simple slope. The test of the simple slope is a t-test with t equal to the simple slope divided by its standard error. The t-test has (n - k - 1) degrees of freedom, where n is the sample size and k is the number of predictors, including the interaction term (Aiken & West, 1991).

Simple slope:

$$\beta_{RNLE} + \beta_{5\text{-HTTLPR*RNLE}} * x$$

$SE_{(\text{simple slope})}$:

$$SQRT(\text{covariance}_{(RNLE)} + 2*x* \text{covariance}_{(RNLE,\ 5\text{-HTTLPR*RNLE})} + x\text{^}2* \text{covariance}_{(5\text{-HTTLPR*RNLE})})$$

t-test:

t = simple slope / $SE_{(\text{simple slope})}$
df = 214 – 3- 1 - 210

Table 4.s1. *covariance matrix*

	5-HTTLPR*RNLE	5-HTTLPR	RNLE
5-HTTLPR*RNLE	1.46	.07	-1.69
5-HTTLPR	.07	1.81	-.10
RNLE	-1.69	-.10	2.67

Fill in values of β (table 4.s2b) and covariances (table 4.s1):

$$t(210) = (3.80 + -2.54 * x) / (SQRT (2.67 + 2 *x* -1.69 + x\text{^}2 * 1.46))$$

Fill in x, which represents 5-HTTLPR coded as 0, 1, or 2 (for low, medium, or high expression groups), and determine the asscoiated p-value:

for x = 0: $t(210) = 2.33$, $p = .021$
for x = 1: $t(210) = 1.45$, $p = .148$
for x = 2: $t(210) = -0.97$, $p = .333$

Based on Aiken & West (1991)

Table 4.s2a. gene environment interactions 5HTTLPR*CEA

		5-HTTLPR				CEA				5-httlpr*CEA				
	IC	*B*	*SE*	*t*	*p*	*B*	*SE*	*t*	*p*	*B*	*SE*	*t*	*p*	r^2
DP positive	1.12	-0.69	3.18	-0.22	0.828	1.01	1.62	0.62	0.534	-0.90	1.09	-0.83	0.409	.004
DP negative	6.32	-5.66	2.86	-1.98	0.049	-0.62	1.45	-0.42	0.673	0.64	0.98	0.65	0.518	.021
RMET total	74.46	0.30	0.85	0.35	0.727	-0.02	0.43	-0.04	0.971	0.17	0.29	0.59	0.554	.006
RMET positive	78.25	1.71	1.50	1.14	0.254	0.25	0.77	0.33	0.743	-0.34	0.52	-0.65	0.519	.009
RMET negative	72.51	-0.60	1.36	-0.44	0.658	0.29	0.70	0.41	0.682	0.22	0.47	0.47	0.643	.012

Table 4.s2b. gene environment interactions 5HTTLPR*RNLE

		5-HTTLPR				RNLE				5-httlpr*RNLE				
	IC	*B*	*SE*	*t*	*p*	*B*	*SE*	*t*	*p*	*B*	*SE*	*t*	*p*	r^2
DP positive	0.85	-0.59	3.17	-0.19	0.853	-3.18	3.81	-0.84	0.405	1.70	2.82	0.60	0.548	.004
DP negative	6.32	-5.61	2.84	-1.97	0.05	-0.03	3.42	-0.01	0.993	1.70	2.53	0.67	0.502	.026
RMET total	74.47	0.33	0.85	0.39	0.701	0.49	1.03	0.48	0.634	-0.55	0.76	-0.72	0.472	.004
RMET positive	78.27	1.67	1.49	1.12	0.264	0.02	1.81	0.01	0.992	-0.89	1.34	-0.66	0.508	.014
RMET negative	72.57	-0.59	1.35	-0.44	0.66	3.80	1.63	2.33	0.021	-2.54	1.21	-2.10	0.037	.026

IC = intercept
DP = Dot Probe task
RMET = Reading the Mind in the Eyes Task

Table 4.s3a. gene environment interactions 5-HTTLPR L_a homozygotes versus S/L_g carriers, and CEA

		5-HTTLPR				CEA				5-httlpr*CEA				
	IC	*B*	*SE*	*t*	*p*	*B*	*SE*	*t*	*p*	*B*	*SE*	*t*	*p*	r^2
DP positive	0.04	0.96	4.94	0.19	0.846	0.57	1.17	0.49	0.625	-1.55	1.71	-0.91	0.365	.004
DP negative	2.27	-6.85	4.46	-1.54	0.126	-0.36	1.05	-0.34	0.732	1.10	1.54	0.71	0.478	.013
RMET total	74.48	1.00	1.33	0.76	0.451	0.12	0.31	0.40	0.693	0.15	0.46	0.34	0.737	.007
RMET positive	78.86	3.81	2.34	1.63	0.106	-0.15	0.55	-0.28	0.782	-0.06	0.81	-0.07	0.944	.013
RMET negative	71.72	0.43	2.13	0.20	0.842	0.43	0.50	0.86	0.389	0.23	0.74	0.31	0.755	.011

Table 4.s3b. gene environment interactions 5-HTTLPR L_a homozygotes versus S/L_g carriers, and RNLE

		5-HTTLPR				RNLE				5-httlpr*RNLE				
	IC	*B*	*SE*	*t*	*p*	*B*	*SE*	*t*	*p*	*B*	*SE*	*t*	*p*	r^2
DP positive	-0.01	0.73	4.93	0.15	0.882	-1.93	2.41	-0.80	0.424	2.14	4.17	0.51	0.609	.003
DP negative	2.31	-6.64	4.43	-1.50	0.136	0.82	2.17	0.38	0.706	3.12	3.76	0.83	0.407	.019
RMET total	74.46	1.10	1.32	0.83	0.408	0.30	0.65	0.47	0.643	-1.31	1.12	-1.17	0.246	.010
RMET positive	78.89	3.71	2.33	1.59	0.113	-0.97	1.15	-0.85	0.397	-0.02	1.98	-0.01	0.994	.017
RMET negative	71.64	0.69	2.10	0.33	0.743	2.35	1.03	2.27	0.024	-4.44	1.78	-2.49	0.013	.034

IC = intercept
DP = Dot Probe task
RMET = Reading the Mind in the Eyes Task