Attentional Bias to Negative Information and 5-HTTLPR Genotype Interactively Predict Students’ Emotional Reactivity to First University Semester

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People strongly differ in their emotional reactions to potentially stressing and challenging environmental circumstances. For example, while some students experience substantial elevations in the frequency of anxiety symptoms during their very first university semester, others show no significant changes or even reductions in anxiety during this period (Clarke, MacLeod, & Shirazee, 2008). Sources of such individual differences have been identified both in the cognitive and in the genetic domain. Specifically, both an attentional bias to negative information, and the short allelic variant of the 5-HT transporter gene linked polymorphic region (5-HTTLPR), have been associated with elevated emotional vulnerability. In the present study, we sought to determine whether these two facets of individual differences independently or interactively contribute to students’ emotional reactivity to their first university semester as a mild and extended potential stressor.

A wealth of research demonstrates that anxious and depressive individuals display a selective attentional bias that favors emotionally negative relative to neutral stimuli (cf., Bar-Haim, Lam, Pergamin, Bakermans-Kranenburg, & van IJzendoorn, 2007; Cisler & Koster, 2010; Mathews & MacLeod, 2005). Assessment of such attentional bias has been carried out using a wide range of cognitive-experimental tasks, in which reaction time (RT) or accuracy measures are typically used to make inferences about attentional allocation (Bar-Haim et al., 2007; Cisler, Bacon, & Williams, 2009; Williams, Mathews, & MacLeod, 1996). One of the most common assessment approaches is the attentional probe task, which requires participants to discriminate the identity of small probes presented in the spatial locus of either negative or neutral stimuli, and reveals attentional bias to negative stimuli by the relative speeding of discrimination latencies for probes presented in the former locus (MacLeod, Mathews, & Tata, 1986; Bar-Haim et al., 2007). Importantly, there is good evidence that attentional bias to negative stimuli makes a causal contribution to emotional vulnerability. For instance, it has been demonstrated...
that an early measure of this attentional bias can predict emotional reactivity to later stressful life-events (Beevers & Carver, 2003; MacLeod & Hagan, 1992). Moreover, such initial measures of attentional bias can predict cortisol responses to subsequent experimental stressors (Dandeneau, Baldwin, Baccus, Sakellaropoulo, & Puressner, 2007; Ellenbogen, Carson, & Pishva, 2010; Fox, Cahill, & Zoukgou, 2010; Pilgrim, Marin, & Lupien, 2010). Furthermore, the experimental manipulations of the attentional bias has been shown to modify negative affect and emotional reactions to stress (Amir, Weber, Beard, Bomyea, & Taylor, 2008; Dandeneau et al., 2007; MacLeod, Rutherford, Campbell, Ebsworthy, & Holker, 2002; Schmidt, Richey, Buckner, & Timpano, 2009; See, MacLeod, & Bridle, 2009). Thus, attentional bias to negative information is widely considered to be an etiological factor in emotional vulnerability.

With respect to biological variables that contribute to emotional vulnerability, growing evidence points to an involvement of the central serotonergic (5-HT) system (Firk & Markus, 2007; Jans, Riedel, Markus, & Blokland, 2007; Lowry et al., 2008). Specifically, it appears that individual differences in emotional vulnerability might be partly caused by variations in genes related to serotonergic transmission. A potential candidate in this respect is a 43 base-pair insertion/deletion polymorphism in the promoter region of the serotonin transporter (5-HTT) gene (Heils et al., 1996; Lesch et al., 1996). This 5-HTT gene linked polymorphic region (5-HTTLPR) comprises two common variants, namely a short (S) and a long (L) allele. Although the functional role of the 5-HTTLPR in serotonergic transmission is not fully understood yet (e.g., Heinz et al., 2000; Lesch et al., 1996; Lim, Papp, Pinsonneau, Sadée, & Saffen, 2006; Parsey et al., 2006; Murthy et al., 2010), the S allele has been repeatedly linked to increased scores in self-reported personality traits associated with negative emotionality, such as neuroticism or harm avoidance (Munafo, Clark, & Flint, 2005; Sen, Burmeister, & Ghosh, 2004). This association has also been observed when using a more implicit measure of personality (Osinisky et al., 2010). Moreover, it has been demonstrated that the S allele is linked to an increased reactivity of limbic sites to emotional stimulation (Munafo, Brown, & Hariri, 2008) and to an enhanced cortisol-response to stress (either alone [Gotlib, Joormann, Minor, & Hallmayer, 2008; Way & Taylor, 2010] or in interaction with lifetime-history of stressful events [Alexander et al., 2009]). Thus, this polymorphism appears to have a substantial impact on basal neuronal and endocrine mechanisms involved in the body’s stress reaction.

There is also evidence that the 5-HTTLPR modulates emotional susceptibility to environmental adversity (Caspi, Harriri, Holmes, Uher, & Moffitt, 2010). In a 23-year longitudinal study, Caspi et al. (2003) found that indices of life-time stress significantly predicts levels of depression and suicidality in carriers of the 5-HTTLPR S allele, but not in homozygous carriers of the L variant. Similarly, it has been found that carriers of the 5-HTTLPR S allele show greater vulnerability to stress in studies of substance-abuse in university students (Covault et al., 2007), and of maltreated children (Kaufman et al., 2007). Carriers of the S allele also show an increased risk for developing a posttraumatic stress disorder and/or major depression, after having been exposed to a devastating natural disaster (Kilpatrick et al., 2007). Accordingly, the S allele has also been identified as contributing to heightened emotional vulnerability, through impairing “ability to maintain healthy and stable levels of psychological functioning in the wake of stress and trauma” (Stein, Cambell-Sills, & Gelernter, 2009, p. 900). However, the 5-HTTLPR genotype not only influences affective reactivity to severe negative life events (e.g., death of a beloved person), but also to mild daily stressors (Gunhert et al., 2007). Although it should be noted that two meta-analyses have generally questioned the link between the 5-HTTLPR S allele and an increased emotional susceptibility (Munafo, Durrant, Lewis, & Flint, 2009; Risch et al., 2009), a more recent and comprehensive meta-analytic approach on this topic provides clear support for such relation (Karg, Burmeister, Shchedden, & Sen, 2010). In sum, there is strong empirical evidence that emotional vulnerability is elevated in carriers of the 5-HTTLPR S allele.

Thus, separate lines of research have revealed that both the 5-HTTLPR and attentional bias to negative information contribute to heightened emotional reactivity to potential stressors. However, it remains unclear whether these two factors make their contributions to emotional vulnerability in an independent, or in an interrelated manner. These genetic and attentional variables might influence emotional reactivity in any of three different ways. First, the 5-HTTLPR and attentional bias potentially could account for different portions of the variance in negative emotional responses to a potential stressor, therefore serving as independent predictors. A second possibility is that the genetic factor may partly exert its influence on emotional vulnerability via its impact on attentional bias. In fact, there is recent evidence that the 5-HTTLPR itself is associated with biased attentional processing of emotional stimuli (Beevers, Gibb, Mcgeary, & Miller, 2007; Beevers, Ellis, Wells, & Mcgeary, 2010; Beevers, Wells, Ellis, & Mcgeary, 2009; Fox, Ridgewell, & Ashwin, 2009; Osinsky et al., 2008; Pérez-Edgar et al., 2007; Thomason et al., 2010). A third possibility is that the genetic and attentional factors may interactively determine emotional vulnerability, such that the 5-HTTLPR modulates the impact of attentional bias on emotional reactivity to a potential stressor. That is, the 5-HTTLPR genotype might contribute to the general arousal of neuro-cognitive modules involved in emotional processing (Canli et al., 2005; Canli & Lesch, 2007; Rao et al., 2007), such that the relation between the attentional bias and sensitivity to a potential stressor will depend upon the 5-HTTLPR genotype.

The present study was designed to empirically evaluate these possibilities, by applying a prospective approach. Following Clarke et al. (2008), we analyzed students’ changes in anxiety and dysphoria across their first semester of tertiary studies. Since the results of Clarke et al. suggest that affective reactions to this potentially stressing and/or challenging life-period strongly differ between individuals, it provides a well-defined time frame across which we can expect individual differences in emotional vulnerability to manifest themselves. We examined the degree to which individual differences in negative emotional reactivity to this mild and extended potential stressor, cross-semester, were predicted by the 5-HTTLPR genotype, and by the pattern of attentional bias revealed by an attentional probe task delivered at the beginning of the semester.

Method

Overview

First year university students, about to experience their potentially stressing initial semester of tertiary studies were invited to
participate in three assessment sessions conducted during the first semester. Participants were genotyped for the 5-HTTLPR: homozygous carriers of the L allele (LL), homozygous carriers of the S allele (SS), and heterozygous participants (LS). In Week 1 of the semester, anxiety and depression levels were assessed, as was attentional bias toward negative information. Participants had their anxiety and depression levels assessed again on Week 2 of semester, and once more on Week 13 of semester. This final assessment took place on the last week of classes, though all participants have exams scheduled during the two weeks subsequent to this assessment.

Participants

Commencing first year students were recruited from the University of Western Australia’s Orientation day, an event providing students with the opportunity to obtain information about the university clubs and services available to them. One hundred and thirty students (83 females; age: \( M = 17.75 \) years, \( SD = 1.56 \)) initially agreed to participate in this study. Ten participants failed to complete all sessions. Thus, the final sample consisted of 120 participant (71 females) with a mean age of approximately 18 years (\( M = 17.7, SD = 1.6 \)). Twenty-seven of these participants were homozygous carriers of the 5-HTTLPR L allele (LL), 62 carried one S and one L allele (SL), and 31 were homozygous carriers of the S variant (SS). This distribution does not significantly deviate from expectations based on the Hardy-Weinberg Equilibrium (\( \chi^2 = .14, df = 1, p = .71 \)). There were no significant differences between the three groups in terms of age, \( F(2, 117) = 1.74, p = .18 \), or gender-distribution (\( \chi^2 = .54, df = 2, p = .76 \)). Participants were reimbursed with $45 for their time and traveling expenses, after completing the final session.

Questionnaire Measures

The Spielberger Trait Anxiety Inventory (STAI-T; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983) was used to measure the frequency of anxiety symptoms. This questionnaire consists of 20 items (4-point format), with total scores ranging between 20 and 80. Higher scores indicate greater frequency of anxiety symptoms. Dysphoria was assessed by means of the Beck Depression Inventory (BDI-II; Beck, Steer & Brown, 1996). This self-report instrument comprises 21 items in a 4-point format which measures depressive symptoms across the preceding two weeks. Scores range from 0–63, with higher scores indicating higher levels of dysphoria.

Dot Probe Task

A dot probe task was used to measure attentional response to negative information on Week 1 of the semester (MacLeod et al., 1986). For this task, the same 96 word pairs employed in the dot probe procedure reported by MacLeod et al. (2002) were used. These word pairs were selected by researchers from an initial pool of 140 word pairs. They were chosen on the basis of 12 judges’ emotion ratings on a 9-point scale, which ranged from very negative (1) to very positive (9). Selection was designed to ensure that the two items in each pair differed in their emotional valence, one being negative and the other neutral. The two members of each word pair were matched for letter length and frequency of usage (according to Kučera & Francis, 1967). An additional 16 pairs of words neutral in emotional valence were constructed for use in practice trials.

The dot probe task consisted of 96 assessment trials, each of which began with the 500 ms presentation of the centered words “Next Trial,” on an otherwise blank screen. Immediately following the termination of this display, a vertically aligned word pair from the stimulus set appeared for 1000 ms. The words were 1 cm in height and formatted in white block text. One member of the word pair appeared directly above, and the other directly below, the center of the monitor. A vertical distance of 3 cm separated these two words, subtending a visual angle of separation of slightly greater than 3° at a viewing distance of 50 cm. Subsequently, this display was replaced by a probe that appeared in either of the two locations vacated by previously presented words. The probes appeared with equal frequency in the locations vacated by either the negative or the neutral word in the preceding pair. Probes consisted of either one small red dot or two closely adjacent red dots, and probe type was random on each trial. Participants were required to discriminate the probe type, and respond by pressing the left mouse button if the probe comprised one red dot, and the right mouse button if the probe comprised two red dots. After this response was detected the screen was cleared, and the next trial began 500 ms later. A relatively long stimulus-onset-asynchrony of 1000 ms between word pairs and probe stimuli was chosen based on previous research demonstrating that only at longer exposure durations is it the case that both elevated levels of anxiety as well as elevated levels of depression are characterized by attentional bias to negative stimuli (e.g., Bar-Haim et al., 2007; Gotlib & Joormann, 2010; Mogg & Bradley, 2005).

On each trial, the delay between probe onset and detection of the participant’s response was recorded, to provide a measure of probe discrimination latency. The rationale behind this task assumes that attentional bias toward negative information will be evidenced by a relative speeding of discrimination latencies for probes in the vicinity of negative words, compared with probes in the vicinity of neutral words. Accordingly, after excluding erroneous (mean error rate = 2.69%, \( SD = 2.44 \)) and extremely slow responses (above the individual’s 95th percentile), an attentional bias index was calculated for each participant, by subtracting their mean discrimination latency for probes in the location of negative words from their mean discrimination latency for probes in the location of neutral words. Higher scores on this attentional bias index were taken to indicate greater attentional bias toward negative information.

Five Acorn Archimedes 410 computers (Acorn Computers Ltd., Cambridge, England), each with a high resolution monitor and an attached mouse, were used to present the stimuli and record the responses. On each mouse, the left and right mouse buttons were labeled with one and two dots, respectively, which indicated the appropriate responses to the corresponding probe identities. Participants were seated in a sound-attenuated cubic approxi- mately 50 cm away from the computer monitor. They were instructed to identify as quickly and as accurately as possible, the identity of the probe that appeared on each trial after the offset of the word pair.
5-HTTLPR Genotyping

Participants were genotyped for the 5-HTTLPR by means of polymerase chain reaction (PCR) and gel electrophoresis. DNA was extracted from buccal cells using a MagNA Pure LC System (Roche, Mannheim, Germany) with a standard commercial extraction kit (High Pure PCR Template Preparation kit; Roche, Mannheim, Germany). Deoxyribonucleic acid amplification reactions were performed using a Mastercycler (Eppendorf, Hamburg, Germany). The detailed procedure is described elsewhere (Osinsky et al., 2008).

Results

Sample Characteristics

Descriptive statistics for STAI-T scores, BDI-II scores, and attentional bias index scores, are provided in Table 1. Note that, across all participants, the mean attentional bias score at Week 1 was small ($M = 4$ ms, $SD = 27$ ms), and did not significantly deviate from zero, $T(119) = 1.54$, $p = .13$. Thus, there was no general tendency in this sample of participants for attention to be systematically shifted toward or away from the location of the negative stimuli. As was the case in Clarke et al.’s (2008) study, anxiety or depression scores did not become consistently elevated over the duration of the semester, across all participants. Rather, there was high variability in cross-semester change in both the anxiety score (mean difference Week 13 minus Week 1 = –0.47 points; $SD = 5.94$) and the dysphoria score (mean difference Week 13 minus Week 1 = 0; $SD = 5.94$), pointing to strong individual differences in the emotional impact of the first semester.

Effect of the HTLLPR Genotype on Attentional Bias

Given that some previous studies have suggested that carriers of the S allele may display an increased attentional bias to negative information, we compared the attentional bias index scores obtained by participants with the differing 5-HTTLPR genotypes. Whether we conducted a binary contrast between those who did or did not carry the S allele (SS and SL vs. LL), or whether we contrasted the three unique genotypes (SS vs. SL vs. LL), no evidence of differential attentional bias was obtained. Although descriptive statistics point to highest attentional biases in SS subjects (see Table 1), group differences were far from significant, allele level: $T(118) = 0.78$, $p = .44$; genotype level: $F(2, 117) = 0.73$, $p = .48$. A significant association between the 5-HTTLPR and attentional bias would be a prerequisite for a mediation-effect of the attentional bias on the relation between the 5-HTTLPR and emotional reactivity (Baron & Kenny, 1986). Therefore, no further mediational analyses were conducted.

Mean-Level Cross-Semester Changes in Anxiety and Dysphoria

Anxiety and dysphoria scores were entered into a 3 (time point: Week 1, Week 2, and Week 13) x 3 (5-HTTLPR genotype: SS, SL, and LL) mixed-model analyses of variance (ANOVA) to assess mean-level changes across the semester.

For anxiety there was a significant main effect of measurement time point, $F(2, 234) = 3.41$, $p = .046$, $\eta^2 = .079$, with pairwise-comparisons indicating a significant decrease from semester Week 1 to Week 2 across all participants ($p < .001$). There was no significant difference in anxiety between Week 1 and Week 13 ($p = .13$) or between Week 2 and Week 13 ($p = .45$). Neither alone, $F(2, 117) = 0.40$, $p = .67$, nor in interaction with the measurement time point, $F(4, 234) = 1.87$, $p = .13$, $\eta^2 = .079$, did the 5-HTTLPR genotype exert an influence on anxiety scores.

Across all participants there were no significant mean-level changes in dysphoria across the semester, $F(2, 234) = 0.01$, $p = .97$, $\eta^2 = .013$. Again, there was no significant effect of the 5-HTTLPR genotype alone, $F(2, 117) = 0.69$, $p = .51$, or in interaction with the measurement time point, $F(4, 234) = 1.79$, $p = .15$, $\eta^2 = .022$.

Individual Cross-Semester Changes in Anxiety and Dysphoria

To quantify emotional reactivity to the first-semester, cross-semester change scores were calculated for anxiety and dysphoria in the manner recommended by Jacobson and Truax (1991), using the formula:

$$\text{individual change score} = \frac{x_1 - x_3}{S_{\text{diff}}},$$

where $x_1$ represents the participants anxiety or dysphoria score at Week 1, $x_3$ represents the participant’s score at Week 13, and $S_{\text{diff}}$ is the standard error of the difference, which can be computed from the standard error of measurement $S_E$ using the equation:

$$S_{\text{diff}} = \sqrt{S_E^2 + S_{\text{diff}}^2},$$

where $S_E$ is the standard deviation for that emotion score at Week 1, and $r_{12}$ is the correlation between the emotion scores at Week 1 and scores at Week 2 (reliability).

The association between the 5-HTTLPR genotype and these individual change scores was then analyzed by means of one-way ANOVAs. Neither for anxiety, $F(2, 117) = 2.26$, $p = .11$, nor for dysphoria, $F(2, 117) = 2.36$, $p = .10$, was a significant effect of 5-HTTLPR genotype on individual cross-semester change detected.

A set of hierarchical moderated regression analyses (Aiken & West, 1991) was then conducted to determine the relationship between attentional bias and individual cross-semester changes in anxiety and dysphoria, and to assess for the potential modulatory impact of the 5-HTTLPR genotype on this relation. Since there were three genotype groups, we calculated three regression analyses for dysphoria and anxiety. In the first step of each regression

\[1\] An anonymous reviewer suggested including only participants with low BDI scores (<12) at semester Week 1 to control for potentially disturbing effects of dysphoria on the relation between the 5-HTTLPR genotype and attentional bias to threat. However, analyses in the restricted subsample ($N = 90$) also did not reveal any significant effects of the 5-HTTLPR on attentional bias scores, neither on the genotype, $F(2, 89) = 1.81$, $p = .17$, nor on the allele level, $T(88) = 0.66$, $p = .51$. 

we entered the attentional bias score and two dummy variables, coding for the 5-HTTLPR genotype (Regression 1: LS = 0/0, LL = 1/0; SS = 0/1; Regression 2: LS = 1/0, LL = 0/0; SS = 0/1; Regression 3: LS = 1/0, LL = 0/1; SS = 0/0). This first step yields a single beta coefficient for the attentional bias score, indicating whether or not attentional bias is a significant predictor for individual changes in anxiety or dysphoria in the sample as a whole. In the second step we entered the product-terms of the attentional bias score and each dummy variable, representing the interaction of genotype and attentional bias. In each of the three regressions this step yields a simple slope for the regression of the individual change score on the attentional bias index for one genotype group (the reference group which is coded 0/0 on the dummy variables). Moreover, betas of the attentional bias × 5-HTTLPR interaction terms indicate whether slopes differ significantly in size between genotype groups. Attentional bias scores were z-standardized before being entered into regressions, and before interaction terms were calculated.

For anxiety, the main results of the moderated regression analyses are listed in Table 2. In regression Step 1, significant variance in cross-semester anxiety changes was explained by the predictors, $R^2 = .07; F(3, 116) = 2.96, p = .04$. Across all participants, the attentional bias index scores significantly predicted individual-level cross-semester changes in anxiety ($B = 0.30, p = .04$).

Specifically, participants with higher attentional bias index scores, indicating relatively greater selective attention to negative information on the first week of the semester, also had a greater tendency to demonstrate subsequent increases of anxiety scores from Week 1 to Week 13. Entering the interaction terms of the 5-HTTLPR genotype × attentional bias index score in Step 2 did not significantly increase the predictive value of the model, $\Delta R^2 = .03; F(2, 114) = 1.74, p = .18$. Moreover, the differences between the slopes of the three genotype groups were not significant (all $p >= .07$). Therefore, the 5-HTTLPR genotype did not modulate the degree to which cross-semester anxiety changes were predicted by initial attentional bias toward negative information.

The results of the moderated regression analysis for cross-semester changes in dysphoria are listed in Table 3. Significant variance in dysphoria score cross-semester change was explained in regression Step 1, $R^2 = .08; F(3, 116) = 3.15, p = .03$. Again, there was a significant positive predictive value of the attentional bias index scores ($B = 0.32, p = .03$). Entering the interaction terms in Step 2 significantly increased the predictive capacity of the model, $\Delta R^2 = .08; F(2, 114) = 5.69, p < .01$, indicating that the capacity of the attentional bias index to predict cross-semester change in dysphoria scores was moderated by the 5-HTTLPR genotype.

The nature of this interactive influence is shown in Figure 1. Comparison of the relative strength of product-terms revealed that the slope of the function, reflecting the relationship between the attentional bias index score and cross-semester change in dysphoria, was significantly greater in SS carriers than in LS carriers (difference in $B = 1.16, p < .01$), and was marginally greater in SS than in LL carriers (difference in $B = 1.08, p = .057$). Indeed, only in SS carriers did this slope significantly deviate from zero ($B = 1.21, p < .01$), whereas this was not so for either LL carriers ($B = 0.13, p = .80$) or SL carriers ($B = 0.05, p = .77$). Thus, only in SS carriers cross-semester dysphoria changes were significantly predicted by the attentional bias index scores. Specifically, SS carriers who showed greater selective attention to negative information on the first week of the semester subsequently showed greater increases in dysphoria scores across the semester, whereas for SL and LL carriers variation in attentional bias index scores did not predict cross-semester elevations in dysphoria scores. Thus, the 5-HTTLPR genotype moderated the degree to which attentional bias to negative information impacted on dysphoric reactivity to the mild extended challenges and potential stressors of participants’ first university semester.

Table 1

<table>
<thead>
<tr>
<th>Variable name</th>
<th>Whole sample</th>
<th>LL</th>
<th>SL</th>
<th>SS</th>
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<tbody>
<tr>
<td>Anxiety Week 1</td>
<td>38.6 (9.3)</td>
<td>39.4 (10.7)</td>
<td>38.7 (9.5)</td>
<td>37.8 (7.9)</td>
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<tr>
<td>Anxiety Week 2</td>
<td>37.5 (9.5)</td>
<td>37.2 (10.2)</td>
<td>37.9 (9.5)</td>
<td>36.8 (9.2)</td>
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<tr>
<td>Anxiety Week 13</td>
<td>38.1 (9.5)</td>
<td>38.0 (9.8)</td>
<td>39.3 (10.1)</td>
<td>36.0 (7.7)</td>
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<tr>
<td>Dysphoria Week 1</td>
<td>9.2 (6.9)</td>
<td>8.3 (6.0)</td>
<td>9.7 (7.6)</td>
<td>8.8 (6.1)</td>
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<tr>
<td>Dysphoria Week 2</td>
<td>9.1 (7.3)</td>
<td>8.6 (5.8)</td>
<td>9.6 (8.1)</td>
<td>8.6 (6.9)</td>
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<tr>
<td>Dysphoria Week 13</td>
<td>9.2 (7.1)</td>
<td>9.5 (7.8)</td>
<td>10.1 (7.0)</td>
<td>7.0 (6.4)</td>
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</table>

Table 2

<table>
<thead>
<tr>
<th>Attentional bias</th>
<th>B</th>
<th>SE in B</th>
<th>T</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>Overall</td>
<td>0.30</td>
<td>0.14</td>
<td>2.06</td>
<td>.04</td>
</tr>
<tr>
<td>Simple slopes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS</td>
<td>0.15</td>
<td>0.17</td>
<td>0.85</td>
<td>.40</td>
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<tr>
<td>Difference in slopes</td>
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<tr>
<td>SL vs. LL</td>
<td>0.06</td>
<td>0.51</td>
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<td>.90</td>
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<tr>
<td>SS vs. LL</td>
<td>0.58</td>
<td>0.57</td>
<td>1.02</td>
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<tr>
<td>SS vs. LL</td>
<td>0.64</td>
<td>0.35</td>
<td>1.85</td>
<td>.07</td>
</tr>
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</table>

Note. Unstandardized betas (B), standard errors (SE), and T and p values are presented. The overall effect indicates whether individual change scores are significantly predicted by the attentional bias in the whole sample. Simple slopes reflect the relation between attentional bias and individual change in anxiety separately for each genotype group. Statistics for difference in slopes indicate whether the size of relation between attentional bias and individual change in anxiety differs significantly between genotype groups.
The main purpose of the present study was to investigate the potential association between two frequently reported sources of individual differences in emotional vulnerability, namely attentional bias toward negative information and the 5-HTTLPR genotype. Specifically, we sought to determine whether these two vulnerability factors exert their influence on emotional reactivity to a potential mild stressor independently of each other, or whether their influence instead is interactive. We addressed this issue by examining the capacity of an attentional bias index, and the 5-HTTLPR genotype, to predict anxiety and dysphoria changes across students’ first university semester as an extended and potentially stressing and challenging time-period for our participants.

First, our results replicate those of Clarke et al. (2008) by showing that there are strong individual differences in students’ emotional reactions to this potentially stressing life-period. While in some participants anxiety and dysphoria substantially increased across the first semester, there were no such changes or even decreases in these two affective dimensions in other students. This finding points to a strong variability in our sample regarding emotional vulnerability. Second, a significant amount of this high variance in cross-semester changes in anxiety and dysphoria can be predicted by individual differences in attentional bias to negative information, recorded at the beginning of the semester. Participants who initially displayed relatively greater attention to negative information subsequently showed relatively greater elevations of anxiety and dysphoria across the semester. Third, the capacity of the attentional bias measure to predict cross-semester elevations in anxiety was independent of the 5-HTTLPR genotype. Importantly, however, the capacity of attentional bias to predict cross-semester elevations in dysphoria was significantly moderated by the 5-HTTLPR genotype. Only for those participants who carried two S alleles did the attentional bias at Week 1 significantly predict cross-semester elevation of dysphoria.

Our findings may hold some important implications for future research on emotional vulnerability and reactivity to potential stressors. First of all, our data clearly support the premise that cognitive and biological factors underpinning emotional vulnerability can combine in a nonadditive manner, rather than exerting their influence independently of each other (e.g., Beck, 2008; Caspi et al., 2010; De Raedt & Koster, 2010). Furthermore, their impact appears to differ depending upon the dimension of emotional vulnerability under consideration. Attentional bias to negative information resulted in all participants being more vulnerable to experience anxiety responses to the first university semester, but resulted in heightened vulnerability to experience dysphoria in response to this potential stressor only in those participants homozygous for the S allele of the 5-HTTLPR. The higher predictive value of the attentional bias for dysphoria-changes in homozygous S allele carriers may result from a generally increased reactivity of these individuals to daily stressors (Gunthert et al., 2007; Sugden et al., 2010). In our study, participants with a SS genotype may therefore have been more sensitive to the challenges of their very first university semester. However, whether these challenges produced an increase or a decrease in dysphoria might then have depended on specific patterns in cognitive processing. Moreover, results of a recent study suggests that the relation between the

**Table 3**

<table>
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<tr>
<th>Attentional bias</th>
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<tbody>
<tr>
<td>Overall</td>
<td>0.32</td>
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<tr>
<td>Simple slopes</td>
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<td>LL</td>
<td>0.12</td>
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</tr>
<tr>
<td>LS</td>
<td>0.05</td>
<td>0.17</td>
<td>0.30</td>
<td>.77</td>
</tr>
<tr>
<td>SS</td>
<td>1.21</td>
<td>0.30</td>
<td>4.02</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Difference in slopes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SL vs. LL</td>
<td>-0.07</td>
<td>0.51</td>
<td>-0.15</td>
<td>.89</td>
</tr>
<tr>
<td>SS vs. LL</td>
<td>1.08</td>
<td>0.56</td>
<td>1.92</td>
<td>.06</td>
</tr>
<tr>
<td>SS vs. LS</td>
<td>1.16</td>
<td>0.35</td>
<td>3.35</td>
<td>&lt;.01</td>
</tr>
</tbody>
</table>

Note. Unstandardized betas (B), standard errors (SE), and T values are presented. The overall effect indicates whether individual change scores are significantly predicted by the attentional bias in the whole sample. Simple slopes reflect the relation between attentional bias and individual change in dysphoria separately for each genotype group. Statistics for difference in slopes indicate whether the size of relation between attentional bias and individual change in dysphoria differs significantly between genotype groups.

**Discussion**

The main purpose of the present study was to investigate the potential association between two frequently reported sources of individual differences in emotional vulnerability, namely attentional bias toward negative information and the 5-HTTLPR genotype. Specifically, we sought to determine whether these two vulnerability factors exert their influence on emotional reactivity to a potential mild stressor independently of each other, or whether their influence instead is interactive. We addressed this issue by examining the capacity of an attentional bias index, and the 5-HTTLPR genotype, to predict anxiety and dysphoria changes across students’ first university semester as an extended and potentially stressing and challenging time-period for our participants.

First, our results replicate those of Clarke et al. (2008) by showing that there are strong individual differences in students’ emotional reactions to this potentially stressing life-period. While in some participants anxiety and dysphoria substantially increased across the first semester, there were no such changes or even decreases in these two affective dimensions in other students. This finding points to a strong variability in our sample regarding emotional vulnerability. Second, a significant amount of this high variance in cross-semester changes in anxiety and dysphoria can be predicted by individual differences in attentional bias to negative information, recorded at the beginning of the semester. Participants who initially displayed relatively greater attention to negative information subsequently showed relatively greater elevations of anxiety and dysphoria across the semester. Third, the capacity of the attentional bias measure to predict cross-semester elevations in anxiety was independent of the 5-HTTLPR genotype. Importantly, however, the capacity of attentional bias to predict cross-semester elevations in dysphoria was significantly moderated by the 5-HTTLPR genotype. Only for those participants who carried two S alleles did the attentional bias at Week 1 significantly predict cross-semester elevation of dysphoria.

Our findings may hold some important implications for future research on emotional vulnerability and reactivity to potential stressors. First of all, our data clearly support the premise that cognitive and biological factors underpinning emotional vulnerability can combine in a nonadditive manner, rather than exerting their influence independently of each other (e.g., Beck, 2008; Caspi et al., 2010; De Raedt & Koster, 2010). Furthermore, their impact appears to differ depending upon the dimension of emotional vulnerability under consideration. Attentional bias to negative information resulted in all participants being more vulnerable to experience anxiety responses to the first university semester, but resulted in heightened vulnerability to experience dysphoria in response to this potential stressor only in those participants homozygous for the S allele of the 5-HTTLPR. The higher predictive value of the attentional bias for dysphoria-changes in homozygous S allele carriers may result from a generally increased reactivity of these individuals to daily stressors (Gunthert et al., 2007; Sugden et al., 2010). In our study, participants with a SS genotype may therefore have been more sensitive to the challenges of their very first university semester. However, whether these challenges produced an increase or a decrease in dysphoria might then have depended on specific patterns in cognitive processing. Moreover, results of a recent study suggests that the relation between the

**Figure 1.** Scatter plots for the relation between cross-semester changes in dysphoria (individual change scores) and the attentional bias at semester Week 1 separated for the three 5-HTTLPR genotype groups.
attentional processing of affective material and the morphology of neuronal circuits involved in cognitive control is higher in S allele carriers compared with LL subjects (Beevers, Pacheco, Clasen, McGearry, & Schnyder, 2010). It could be that in SS carriers in our study such tightened coupling between attentional bias and cognitive control mechanisms served to increase the influence of the attentional bias on the dysphoric reactivity in these individuals. Related to this topic, results of two recent studies indicate that SS carriers are generally more sensitive to cognitive-affective interventions than L allele carriers (Eley et al., in press; Fox, Zougkou, Ridgwell, & Garner, 2011).

It is interesting to note that we found no differences between 5-HTTLPR genotype groups in terms of attentional bias toward negative information. At first glance, this may seem to conflict with previous findings, including some from our own group, showing an association between 5-HTTLPR and attentional bias (e.g., Beevers et al., 2007; Beevers et al., 2009; Beevers et al., 2010; Fox et al., 2009; Osinsky et al., 2008; Pérez-Edgar et al., 2010; Thomason et al., 2010). However, a closer inspection of the literature reveals some degree of inconsistency that may, in particular, result from differences in methodological aspects of previous work. For instance, prior studies have differed in terms of the paradigms used for attentional bias assessment (e.g., eye tracking or dot probe task), in stimulus presentation latencies (e.g., subliminal, hundreds of milliseconds, or several seconds), and in stimulus material (e.g., complex visual scenes, animals, faces, or words). These differences between studies make comparisons difficult. More research is needed to determine whether such methodological differences systematically modulate the relationship between the 5-HTTLPR and attentional bias.

The possibility that the 5-HTTLPR SS genotype may be associated with an attentional bias that reflects reduced attention to positive information (e.g., Fox et al., 2009), rather than increased attention to negative information, raises issues of potential relevance to the present line of work, which could usefully be addressed in future research. Our own attentional bias assessment task presented only negative-neutral stimulus pairs, and so it assessed only selective attentional response to negative information. It would be informative to also include positive-neutral stimulus pairs in this type of study, in order to distinguish attentional bias to positive and negative emotional information. If the SS genotype is linked to a cognitive bias that involves reduced attention to positive information, then this may go some way toward explaining the fact that its impact was evident on cross-semester elevation of depression scores, but not anxiety scores. There is good evidence that anxiety is characterized by heightened negative affectivity alone, while depression instead is characterized by the co-occurrence of heightened negative affectivity and attenuated positive affectivity (e.g., Lee, Watson, & Mineka, 1994). It is not implausible that heightened attention to negative information may elevate negative affectivity without reducing positive affectivity, whereas reduced attention to positive information instead may serve to attenuate positive affectivity. Hence, if the homozygous SS genotype were characterized by reduced attention to positive information, it would not predict anxiety reactivity, which instead would be predicted by heightened attention to negative information. However, when coupled with such heightened attention to negative information, then the SS genotype could increase susceptibility to dysphoria, by combining impaired attention to positive information with greater attention to negative information, in a manner that combines attenuated positive affectivity with elevated negative affectivity.

Whatever the mechanism underlying its emotional impact, the present pattern of findings suggests that the S allele is recessive, in that its impact was evident only in participants homozygous for this allele. This, too, has been a contentious issue in previous research, and prior findings have been sufficiently inconsistent to ensure that there is no consensus concerning whether the S allele is dominant or recessive with respect to the attentional bias. In the study of Beevers et al. (2009), for example, results of experiment one suggested that the S allele was dominant, while the results of experiment two suggested that it was recessive. Indeed, it is even possible that the L allele may play an active role, by promoting emotional resilience. Our own findings, for example, could be interpreted as evidence either that the S allele expresses emotional vulnerability in a recessive manner, or that the L allele expresses emotional reliance in a dominant manner. Consistent with the possibility that the L allele may play an active role in emotional regulation, Fox et al. (2009) argue that it is the L allele, rather than the S allele, that is associated with selective attentional response to emotional stimuli. Certainly more research is needed to better understand the detailed impact of the 5-HTTLPR genotype on the neuronal foundations of selective attentional processing of emotional material.

In our argumentation so far, we have conceptualized the attentional bias toward negative information as a relatively stable construct. However, there is also evidence that the magnitude of shifts in attentional bias, especially following acute negative-mood inductions, can prospectively predict changes in negative affect later on (Beevers & Carver, 2003; Cavanagh, Urry, & Shin, 2011). Since our study design does not provide measures for such individual differences in the reactivity of attentional bias to acute mood induction, future studies are needed to clarify whether the capacity of such reactive shifts in attentional bias to predict emotional vulnerability also is moderated by the 5-HTTLPR genotype.

Some limitations of the present study should be noted. First, we only used a supraliminal presentation latency (1000 ms) for the emotional-neutral word pairs in our dot probe task. While this specific presentation time was chosen to tap both anxiety- and dysphoria-linked patterns of affective-attentional biases (cf. Bar-Haim et al., 2007; Gotlib & Joormann, 2010; Mogg & Bradley, 2005), we cannot make any inferences about more automatic stages of attentional selectivity. To assess automatic capture of attention by negative stimuli would require much briefer stimulus presentation durations (e.g., 20 ms). Thus, it is not clear whether individual differences in automatic attentional biases toward negative stimuli, which appears to be more strongly characteristic of anxiety vulnerability than depression vulnerability (cf. Bar-Haim et al., 2007), might have differentially predicted cross-semester changes in anxiety depending on the 5-HTTLPR genotype. Moreover, our attentional assessment task did not permit us to distinguish individual differences in attentional engagement with, and attentional disengagement from, negative information (Koster, Crombez, Verschueren, & De Houwer, 2004). Although it was not our own objective, future studies might benefit from using attentional assessment tasks designed to differentiate such aspects of attentional selectivity (e.g., Fox, Russo, Bowles, & Dutton, 2001; Koster et al., 2004). A further limitation of our study is that we
have not assessed an A→G single nucleotide polymorphism (rs25531) within the L allele which has also been demonstrated to influence the 5-HTT gene expression (e.g., Praschak-Rieder et al., 2007; Reimold et al., 2007). However, results of several prior studies on emotional vulnerability question the incremental effect of the tri-allelic 5-HTTLPR classification including this SNP above the “classic” biallelic distinction between S and L allele (e.g., Alexander et al., 2009; Gotlib et al., 2008; Lonsdorf et al., 2009; Pezawas et al., 2008). Moreover, no data on the ethnicity of participants were collected so it is unclear whether this factor has a modulatory influence on the observed effects. Furthermore, for a “candidate gene” approach the size of our sample is rather small, limiting the statistical power for the detection of potentially small 5-HTTLPR effects.

Despite these limitations, we can draw several conclusions from the present study, which we believe further our understanding of the association between 5-HTTLPR, attentional bias, and emotional vulnerability. We can conclude that (a) an initial measure of attentional bias to negative information predicts both anxiety and dysphoric reactions to the extended mild challenges and potential stressors of the very first university semester. Moderator analyses yielded that, (b) for anxiety, the 5-HTTLPR does not significantly influence the predictive capacity of the attentional bias but (c) does so for dysphoric reactions. Only in SS carriers, and not in LS or LL carriers, is it the case that attentional bias to negative information predicts changes in students’ dysphoria across the first semester. Hence, at least one of the ways in which 5-HTTLPR can influence emotional vulnerability is by moderating the degree to which cognitive vulnerability factors, such as attentional bias, exert an impact on peoples’ dysphoric reactions to potentially challenging and stressing environmental circumstances.

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