Research Article

ANXIETY SENSITIVITY IN ADOLESCENCE AND YOUNG ADULTHOOD: THE ROLE OF STRESSFUL LIFE EVENTS, *5HTTLPR* AND THEIR INTERACTION

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Background: *Cognitive biases have long been hypothesized to influence the development and maintenance of symptoms of internalizing problems. Anxiety sensitivity represents one such bias and refers to sensitivity to the physical and emotional symptoms of anxiety and the belief that these are harmful. Twin studies indicate a role for both environmental and genetic influences on anxiety sensitivity. However, little work has been done specifying environments or genes involved in this phenotype. In light of this, we looked at the association between stressful life events, the serotonin transporter gene polymorphism (5HTTLPR), and anxiety sensitivity in a longitudinal sample of adolescents.* **Methods:** *Stressful life events and anxiety sensitivity were measured in over 1,500 individuals at three time points (mean ages 15, 17, and 20 years). 5HTTLPR was genotyped in 1,109 participants.* **Results:** *There was consistent evidence for an association between stressful life events and both anxiety sensitivity and change in anxiety sensitivity over time. Although the effect of independent stressful life events was relatively short lived, dependent stressful life events were associated with anxiety sensitivity over time. There was no evidence for a main effect of 5HTTLPR on anxiety sensitivity. 5HTTLPR genotype did not moderate the effect of stressful life events on anxiety sensitivity.* **Conclusions:** *The current study extends previous work by showing that stressful life events, independent of the individual, explained change in cognitions associated with anxiety and depression. This effect does not, however, appear to be moderated by genotype. Depression and Anxiety 29:400–408, 2012.* © 2012 Wiley Periodicals, Inc.

Key words: *anxiety; gene–environment interaction; cognitive biases; serotonin transporter polymorphism; environmental adversity*

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INTRODUCTION

Anxiety sensitivity refers to hypersensitivity to anxiety-related sensations, and the belief that they have negative social, psychological, or physical consequences.[1] Anxiety sensitivity has not only been shown to be elevated in people with anxiety disorders compared to healthy controls[2] but longitudinal studies have shown that it is also predictive of anxiety over time.[3, 4] Moreover, anxiety sensitivity has been shown to predict subsequent depression.[5, 6] Individual differences in anxiety sensitivity are hypothesized to emerge from the combined influence of genetic variation along with experiences that lead to the acquisition of beliefs about the potentially aversive consequences of anxiety-related states.[7, 8] Given the close links between anxiety sensitivity and both anxiety and depression, it is important to further

understand the developmental etiology of this cognitive bias.

Environmental influences have been shown to be largely responsible for change in anxiety sensitivity over time.[9] However, in stark contrast to the literature on the effect of stressful life events and depression; that on anxiety in general, and anxiety sensitivity in particular is very minimal. In the only previous study to specifically examine stressful life events, they were found to be associated with subsequent increases in anxiety sensitivity.[10] Events relating to health and family discord were particularly pertinent. This study did not, however, address the extent to which the stressful life events were independent of the individual. It is, therefore, plausible that these findings are confounded by characteristics of the individual such as current mood state that subsequently lead an individual to report or experience more stressful life events (e.g. breaking up with a boyfriend/girlfriend).

In addition to evidence implicating environmental influences in the development of anxiety sensitivity, research using the twin design has highlighted the contribution of genes.[9, 11, 12] Moreover, studies have provided evidence to suggest that the genetic influences on anxiety sensitivity are largely shared with those of anxiety and depression.[12, 13] No specific genes have, however, been associated with anxiety sensitivity. Given the high genetic correlations between anxiety sensitivity, anxiety, and depression, a strong candidate gene would be the serotonin transporter gene promoter polymorphism (*5HTTLPR*) that has been associated with both anxiety and depression (for review see [14]). The main effect of this polymorphism has been mixed. Some studies have found evidence of a positive association between the short "S" allele (associated with reduced serotonin transporter expression) and increased affective symptoms (e.g. [15, 16]), whereas others have reported an association between the "L" allele (associated with increased serotonin transporter expression) and anxiety-related traits.[16, 17] Moreover, some studies have failed to find any association between *5HTTLPR* and anxiety-related phenotypes (e.g. [18]).

A growing body of evidence suggests that genetic variation, for example, *5HTTLPR* genotype, might moderate the effect of environmental factors, such as stressful life events (for review see [19]). A seminal study in this area found that individuals with one or two copies of the "S" allele exhibited more depressive symptoms, diagnosable depression, and suicidality in high stress environments than individuals homozygous for the long ("L") allele.[20] This finding has been controversial, and led to numerous replication attempts, some of which have not been successful.[21] A recent meta-analysis found strong evidence that *5HTTLPR* moderated the relationship between stress and depression.[22] The moderation effect remained when studies were grouped into those in which the stressors were stressful life events versus childhood maltreatment or stress caused by specific medical conditions. To date (to our knowledge), there have only been two published studies of gene–environment inter-

actions in anxiety sensitivity.[23, 24] In both studies, results showed that *5HTTLPR* genotype moderated the effect of childhood emotional maltreatment on anxiety sensitivity. However, the direction of effect varied across the two studies. In the first study, individuals with the "SS" genotype and high levels of maltreatment had greater anxiety sensitivity than individuals with the "LL" genotype.[23] Although in the second study, an interaction was observed between the "LL" genotype and childhood maltreatment on anxiety sensitivity.[24] No study to date has examined the interplay between *5HTTLPR* and stressful life events on anxiety sensitivity.

In the current study, we examined three questions. First, we explored the relationship between stressful life events and anxiety sensitivity. We predicted that stressful life events would be positively associated with anxiety sensitivity and could also predict change in anxiety sensitivity over time. Second, we investigated whether *5HTTLPR* was associated with anxiety sensitivity. In line with studies of internalising problems, we hypothesized that the "SS" genotype would be associated with greater anxiety sensitivity. Finally, we investigated whether the association between anxiety sensitivity and stressful life events was moderated by *5HTTLPR*. We predicted that the "SS" genotype would moderate the effect of stressful life events on anxiety sensitivity. The interplay between genes and environment may be particularly pertinent in adolescence where individuals are engaging in more risk taking and sensation-seeking behaviors as well as being increasingly conscious of themselves and their peer environment.[25]

METHOD

PARTICIPANTS

Data from the G1219 sample were used.[26, 27] The G1219 study is a longitudinal study of 1,820 adolescent twin and sibling pairs (age range 12–19 years at initial contact). Contact invitations included questionnaires to be completed by adolescents and their parents at Wave 1. At Wave 2, approximately 8 months after initial contact, data were available from 2,651 individuals (73% of the twin and sibling pairs recruited at Wave 1), whereas corresponding figures for Wave 3, approximately 25 months after Wave 2, were 1,597 adolescents (44% of the twin and sibling pairs at Wave 1). At Wave 4, we traced participants who had taken part in either Wave 2 or Wave 3 primarily by using websites/databases dedicated to providing information (e.g. phone numbers and postal addresses) about members of the population. We successfully traced 2,550 individuals of whom 1,556 responded (61% of those targeted; 74% of those participating at Wave 3). The current investigation uses data from the second, third, and fourth wave of data collection and will be referred to as time 1, 2, and 3, respectively from here on for ease of presentation. Mean age and range (in parenthesis) at times 1, 2, and 3 was 15 years (12–21), 17 years (14–23), and 20 years (18–27), respectively. Informed consent was obtained from parents/guardians of all adolescents under 16 years, and from the adolescents themselves when 16 years or over. Ethical approval for different stages of this study has been provided by the Research Ethics Committees of the Institute of Psychiatry, South London and Maudsley NHS Trust, and Goldsmiths, University of London.

MEASURES

Anxiety Sensitivity. Anxiety sensitivity was measured at time 1 and 2 using the Child Anxiety Sensitivity Index (CASI:[28]), designed for use with children and adolescents. The CASI is an 18-item questionnaire that requires children to rate their level of fear to the same types of anxiety-related sensations or experiences that are represented on the adult version. Participants rate each item on a 3-point likert scale (1 = *none* to 3 = *a lot*). A total CASI score can be computed by summing the items. The CASI has good psychometric properties.[28] In the current sample, internal consistency (assessed by Cronbach's α) at times 1 and 2 was .82 and .86, respectively. By time 3, as participants were all 18 years or older, anxiety sensitivity was assessed using the Anxiety Sensitivity Index (ASI:[29]). This consists of 16 items with participants rating their level of agreement on a 4-point likert scale (from 1 = *very little* to 4 = *very much*). Total ASI scores are, like the CASI, computed by summing the items. The ASI has sound psychometric properties[30] and extensive validity estimates (see[31]). In the current sample, internal consistency of the ASI at time 3 was .87.

Stressful Life events. Participants were asked about stressful life events that had occurred in the past year at times 1 and 2 using a 24-item version of the Life Event Scale for Adolescents (LES-A),[32] and at time 3 (participants over 18 years), using the List of Threatening Experiences (LTE:[33]), supplemented with nine items from the LES-A[32] (see Appendix 1 for full list of events). At time 2, the parents also completed the 24-item LES-A with respect to their adolescent offspring. All events were classified as dependent or independent. This distinction is made according to whether the event is likely to arise from an individual's behavior; thus, "death of a parent" is an example of an independent event, whereas "breakup with a boy/girlfriend" is an example of a dependent event. This distinction is common within the life events literature[34] and has been used in previous studies (see[35]). One of the benefits of distinguishing between dependent and independent life events is that the direction of effects for independent life events is clearer as they are unlikely to be confounded by individuals' characteristics or current mood state. At times 1 and 2, items were split into 12 dependent and 12 independent events. At time 3, items were subdivided into 13 dependent and eight independent events.

Genotyping. DNA from buccal swabs was collected from a total of 1,237 individuals. Participants' ethnicity was not recorded, however, 97.6% of participants' parents stated their ethnicity as white at initial contact. Of these samples, 1,109 were successfully genotyped for *5HTTLPR* (90% call rate). Recently, an A/G single nucleotide polymorphism (SNP) has been identified within the LPR allele.[36] The L_G and S alleles have been reported to have comparable levels of *5HTT* expression that are lower than that of the L_A allele.[36] Given this, we performed a multiplex polymerase chain reaction (PCR) procedure, as outlined by Wendland et al.,[37] which simultaneously genotypes *5HTTLPR* and the A/G SNP within the LPR at the VNTR at intron 2 and the missense mutation I425V. The PCR procedure was followed by double restriction endonuclease digestions using HpaII and BccI, which identifies the A/G SNP within the LPR and the I425V mutation, respectively. Triallelic genotypes were transformed into a biallelic model according to their level of expression as follows: L_GL_G, L_GS, and SS were designated as S'S'; L_AS and L_AL_G as L'S'; and L_AL_A as L'L' (see[38]).

STATISTICAL ANALYSIS

Statistical analysis was carried out in three stages. First, the effects of dependent and independent stressful life events on anxiety sensitivity and *change* in anxiety sensitivity were examined. Second, we tested whether there was any evidence for a main effect of *5HTTLPR* genotype on anxiety sensitivity. Finally, we tested possible interactions between *5HTTLPR* and stressful life events on anxiety sensitivity and change in anxiety sensitivity over time.

Linear regression analysis was employed to test main effects as well as the interaction between *5HTTLPR* and stressful life events on anxiety sensitivity at times 1, 2, and 3. Analyses were conducted in STATA[39] and as our sample included individuals from the same families, statistical analyses were conservatively corrected for the non-independence of observations using the *"robust" cluster* command as is standard in analyses of this type (see[40,41] for more information). Chi-square tests were used to assess associations between measures of stressful life events and genotype. Power calculations using QUANTO revealed, based on analysis by Stein et al., 2008, that there was approximately 73% power to detect a main effect of *5HTTLPR*, 99% power to detect an environmental effect, and 58% power to detect an interaction between them (assuming a main effect of environment).

RESULTS

ANXIETY SENSITIVITY

Levels of anxiety sensitivity decreased over time in the current sample. Mean scores and standard deviations (in parenthesis) at times 1 and 2 were 29.03 (5.32) and 25.64 (5.59), respectively. At time 3, where the ASI was used, the mean and standard deviation was 15.52 (9.08). There was a significant effect of gender (females higher than males), but not age, on anxiety sensitivity at each wave. Sex was therefore included as covariates in all regression analyses. Anxiety sensitivity was moderately correlated over time ($r = .47$ and $r = .38$ between time 1, and times 2 and 3, respectively; $r = .50$ between times 2 and 3).

STRESSFUL LIFE EVENTS AND ANXIETY SENSITIVITY

The mean number (and standard deviation in parenthesis) of dependent stressful life events reported by participants was 1.24 (1.43), 1.25 (1.38), and 1.25 (1.57) at times 1, 2, and 3, respectively. The mean number of independent stressful life events reported by participants was .65 (.94), .69 (.98), and .60 (.86) at times 1, 2, and 3, respectively. Parents reported a mean of .71 (1.00) dependent and .58 (.88) independent stressful life events at time 2. However, consistent with previous studies that provide data on stressful life events, the number of events endorsed was positively skewed and categorizing the variable was the most appropriate way to analyze the data. In the current analysis, stressful life events, both dependent and independent, were categorized into those reporting no, at least one, or two, or more stressful life events (see Table 1). There was a moderate phenotypic correlation between parent- and self-reported dependent ($r = .45$) and independent ($r = .54$) and stressful life events at time 2.

We found evidence for a main effect of dependent stressful life events on measures of anxiety sensitivity over time (see Table 2). For example, dependent stressful life events at time 1 were significantly associated with anxiety sensitivity at time 1 ($\beta = .21$, $P < .01$), as well anxiety sensitivity at both times 2 ($\beta = .17$, $P < .01$) and 3 ($\beta = .14$, $P < .01$). The strength of the relationship, however, decreased over time. Independent stressful life events, conversely, were only associated with proximal

TABLE 1. Number of stressful life events reported

		Number of stressful life events		
		0	1	≥2
Self-rated stressful life events				
Dependent	Time 1	1,041 (40%)	713 (27%)	871 (33%)
	Time 2	572 (39%)	401 (27%)	499 (34%)
	Time 3	641 (42%)	410 (27%)	478 (31%)
Independent	Time 1	1,522 (58%)	684 (26%)	419 (16%)
	Time 2	819 (55%)	420 (28%)	233 (16%)
	Time 3	874 (57%)	479 (31%)	176 (12%)
Parent-rated stressful life events				
Dependent	Time 2	732 (54%)	389 (29%)	223 (17%)
Independent		809 (60%)	375 (28%)	162 (12%)

Note: Number of stressful life events reported for adolescents who had completed both the life events questionnaire and anxiety sensitivity questionnaire at each wave ($N = 2,625$, $N = 1,474$, and $N = 1,529$ for times 1, 2, and 3, respectively).

TABLE 2. Associations between dependent and independent stressful life events and anxiety sensitivity

		Anxiety sensitivity		
		Time 1	Time 2	Time 3
Self-rated stressful life events				
Dependent	Time 1	$\beta = .21^{**}$	$\beta = .17^{**}$	$\beta = .14^{**}$
	Time 2	-	$\beta = .28^{**}$	$\beta = .19^{**}$
	Time 3	-	-	$\beta = .25^{**}$
Independent	Time 1	$\beta = .13^{**}$	$\beta = .08$	$\beta = .04$
	Time 2	-	$\beta = .15^{**}$	$\beta = .04$
	Time 3	-	-	$\beta = .25^{**}$
Parent-rated stressful life events				
Dependent	Time 2	-	$\beta = .12^{**}$	$\beta = .11^{*}$
Independent		-	$\beta = .12$	$\beta = -.01$

Note: $^{*}P < .05$; $^{**}P < .01$. Within time association (e.g. time 1 dependent life events with time 1 anxiety sensitivity); $N = 2,625$, $N = 1,474$, and $N = 1,529$ for times 1, 2, and 3, respectively. For all longitudinal associations (e.g. time 1 dependent life events with time 2 anxiety sensitivity); $N = 1,116$.

TABLE 3. Association between independent and dependent stressful life events and *change* in anxiety sensitivity over time

		Anxiety sensitivity (controlling for time 1 anxiety sensitivity)		Anxiety Sensitivity (controlling for time 2 anxiety sensitivity)
		Time 2	Time 3	Time 3
Self-rated stressful life events				
Dependent	Time 2	$\beta = .21^{**}$	$\beta = .13^{**}$	$\beta = .06$
	Time 3	-	$\beta = .21^{**}$	$\beta = .16^{**}$
Independent	Time 2	$\beta = .08^{***}$	$\beta = -.04$	$\beta = -.04$
	Time 3	-	$\beta = .11^{*}$	$\beta = .10^{*}$
Parent-rated stressful life events				
Dependent	Time 2	$\beta = .05$	$\beta = .05$	$\beta = .04$
Independent		$\beta = .05$	$\beta = -.04$	$\beta = -.05$

Note: $^{*}P < .05$; $^{**}P < .01$; $^{***}P = .05$. $N = 1,116$.

measures of anxiety sensitivity. For example, time 1 independent stressful life events were associated with time 1 anxiety sensitivity ($\beta = .13$, $P < .01$), but not anxiety sensitivity at either times 2 ($\beta = .08$, $P > .05$) or 3 ($\beta = .04$, $P > .05$). For parent-reported events, only those of a dependent nature were associated with anxiety sensitivity.

We then went on to look at whether stressful life events predicted *change* in anxiety sensitivity over time by controlling for earlier ratings of anxiety sensitivity (see Table 3). Dependent stressful life events consistently predicted change in anxiety sensitivity over time. For example, time 2 dependent stressful life events significantly predicted change in anxiety sensitivity from time 1 to time 2 ($\beta = .21$, $P < .01$) and from time 1 to time 3 ($\beta = .21$, $P < .01$). The latter being particularly interesting as ratings of stressful life events and anxiety sensitivity were collected at different times. For

independent stressful life events, only those at time 3 predicted change in anxiety sensitivity (from times 1 and 2 to time 3). Finally, we investigated the cumulative effects of stress over development on anxiety sensitivity at time 3 by combining stressful life events at times 1, 2, and 3. Results showed that repeated exposure to both dependent ($\beta = .13$, $P < .01$) and independent ($\beta = .06$, $P < .01$) stressful life events predicted anxiety sensitivity at time 3. Change in anxiety sensitivity from time 2 to time 3 was predicted by the cumulative effects of dependent stressful life events ($\beta = .06$, $P < .01$) but not independent stressful life events ($\beta = .01$, $P > .05$).

5HTTLPR AND ANXIETY SENSITIVITY

Participants were more likely to provide DNA if they reported more life events at time 1 (time 1; $\chi^2 = 85.17$ $P < .01$) but there was no significant difference in number of life events reported at time 2 ($\chi^2 = 0.96$ $P > .05$) or time 3 ($\chi^2 = 0.11$ $P > .05$). A genotypic model (L'L', L'S', S'S') was used to examine the effects of *5HTTLPR* on anxiety sensitivity. Distributions of genotype were in hardy–weinberg equilibrium (HWE test: χ^2(1DF) < .001, $P = .996$). The number of participants, along with the mean anxiety sensitivity score, across each level of genotype and stressful life events are shown in Appendix 2. We found no evidence for a main effect of *5HTTLPR* genotype on anxiety sensitivity (see Appendix 3).

GENE–ENVIRONMENT INTERACTION

We tested for gene–environment correlation as this can increase type I error rates (see[42]). However, we found no association between self-reported dependent or independent stressful life events and *5HTTLPR*. We, therefore, went on to test possible gene–environment interactions using the genotypic model and 3 level stressful life events variable (described previously). *5HTTLPR* did not moderate the effect of stressful life events, either dependent or independent, on anxiety sensitivity or *change*

in anxiety sensitivity over time (results presented in Appendix 3). Moreover, the cumulative effects of stressful life events on anxiety sensitivity were not moderated by variation in *5HTTLPR* genotype. There was some evidence of a moderation effect of *5HTTLPR* on the association between parent-reported independent stressful life events and anxiety sensitivity ($\beta = -.17$, $P < .05$). This did not survive Bonferroni correction and is likely to be a result of multiple testing.

DISCUSSION

SUMMARY

Anxiety sensitivity has consistently been shown to be an important risk factor for both anxiety and depression.[4, 6, 43] The purpose of this study was to investigate the effect of dependent and independent stressful life events on anxiety sensitivity, and to examine the extent to which these effects are moderated by *5HTTLPR* genotype.

Both dependent and independent stressful life events were associated with higher anxiety sensitivity ratings as well as change in anxiety sensitivity over time. This is in line with the only previous article that has suggested a role for stressful life events in the development of anxiety sensitivity.[10] Our results are also supported by our previous twin analyses using data from the G1219 sample that suggest that environmental influences are largely responsible for change in anxiety sensitivity over time.[9] There was no evidence of a main effect of *5HTTLPR* on anxiety sensitivity and we failed to replicate previous findings suggesting that the effect of environmental stress on anxiety sensitivity is moderated by *5HTTLPR*.

STRESSFUL LIFE EVENTS AND ANXIETY SENSITIVITY

We conducted analyses with dependent and independent life events separately as the effect of dependent life events may be confounded by characteristics of an individual that might lead them to experience or report more stressful life events. So although dependent stressful life events were associated with higher anxiety sensitivity both proximally as well as longitudinally, the direction of effects is unclear. For example, although it is possible that dependent life events lead to increases in anxiety sensitivity, it is also plausible that those with higher anxiety sensitivity report or even elicit a greater number of dependent events. However, the finding that dependent life events lead to *change* in anxiety sensitivity suggests that the direction of effects goes from dependent stressful life events to anxiety sensitivity.

Independent stressful life events were only associated with proximal assessments of anxiety sensitivity, suggesting that they have a relatively short-lived effect. This is in line with much of the stressful life events literature relating to depression. Such research tends to find that the influence of stressful life events on mood diminishes after 6 months.[19, 34] Independent stressful life events also predicted *change* in anxiety sensitivity over adolescence, although the effect was relatively modest. Stressful life events, particularly those that occur "out of the blue," might have an effect on anxiety sensitivity by, for example, activating schemas relating to threat and danger causing the individual to have trouble shifting attention and activating or inhibiting behavior that would lead to a decrease in negative affect and arousal. This would consequently increase anxiety sensitivity, as individuals would become hypersensitive to anxiety and anxiety-related sensations. Beliefs about the negative consequences of these anxiety symptoms would also be reinforced.

The effect of stressful life events on change in anxiety sensitivity is in line with similar research on rumination, a cognitive bias associated with the maintenance of depression.[44, 45] Rumination involves repetitively focusing on the symptoms of distress, and on its possible causes and consequences. Rumination and anxiety sensitivity, therefore, share features such as the focus on the interpretation of and consequences of distress.[10] It makes sense, therefore, that cognitive biases that share key features, in this case the level of self-focused thought, will be elicited by similar environmental stressors.

5HTTLPR, STRESSFUL LIFE EVENTS, AND ANXIETY SENSITIVITY

There was no evidence of a main effect of *5HTTLPR* on anxiety sensitivity, in line with previous research.[23, 24] However, although the results of the current study and previous research suggest that *5HTTLPR* does not directly affect anxiety sensitivity, further replication would be required before any firm conclusion can be made. This is because of the association between anxiety sensitivity and anxiety and the strong genetic overlap between these phenotypes in quantitative genetic studies and power limitations associated with the current study.

Although there are several reasons why gene–environment interactions are expected in psychopathology,[46] the current study failed to support previous evidence for an interaction between *5HTTLPR* and environmental adversity predicting anxiety sensitivity.[23, 24] There are several possible reasons for this disparity (for a review see[19]). First, we investigated the moderating effects of *5HTTLPR* on stressful life events and anxiety sensitivity in an adolescent sample (the previous analyses were conducted on older samples). The effect of *5HTTLPR* might be developmentally sensitive and thus explain why we have failed to replicate findings from an adult sample. There is little research suggesting a developmentally sensitive effect of this interaction, although some studies in adolescence have shown that the interaction between stressful life events and *5HTTLPR* is limited to females (e.g.[47]). In the current study, environmental adversity

was identified by the number of stressful life events endorsed. Previous research has suggested that the interaction is stronger when stressful life events are assessed by interview than questionnaire.[19, 48] Moreover, the only previous articles on gene–environment interactions in anxiety sensitivity used retrospective reports of childhood maltreatment as a measure of adversity.[23, 24] Although a recent meta-analysis showed the interaction between *5HTTLPR* and stress on depression is not specific to a particular environment, the interaction was stronger for childhood maltreatment than stressful life events.[22]

Finally, power calculations suggest that a sample of 1,700 is required to have 80% power to detect an interaction effect. The current study had 58% power and was, therefore, relatively underpowered.

Furthermore, stressful life events were measured using questionnaires that were quick and easy to complete. While facilitating the collection of data from a large sample of participants, enquiring about specific stressful life events, means that some events occurring during the index periods may be missed. In addition, although stressful life events were categorized into independent and dependent events, a structured interview such as the Life Events and Difficulties Schedule (LEDS)[34] would be required to establish true independence of events. More recently, studies have also suggested extending analyses to examine a wider array of proximal environmental measures, for example, family and peer relationships.[48]

CONCLUSION

Overall, the finding that stressful life events predicted *change* in anxiety sensitivity during adolescence suggests that stressful life events affect an individual's cognitions. That an association was found between *independent* stressful life events and *change* in anxiety sensitivity over time suggests the relationship is not solely due to characteristics of the individual that are leading them to experience more life events. Although a previous study found evidence of a moderation effect of *5HTTLPR* on anxiety sensitivity in the context of environmental stress, this was not supported in the current study. Further replication is required as there is not adequate power in the current study to reject the null hypothesis. The current study extends previous work that has shown that stressful life events predict increases in internalizing symptoms,[34] by showing that such events are also important in explaining change in cognitions associated with anxiety and depression.

APPENDICES

APPENDIX 1. List of stressful life events asked at times 1–3

Times 1 and 2	Time 3
Dependent stressful life events	
Becoming involved with drugs	Separation due to marital difficulties
Being sent away from home	Serious problem with close friend, neighbour, or relative
Failing to achieve something you really wanted	Problems with police or court appearance
Appearance in a juvenile court	Unemployed or seeking work for more than a year
Start of a new problem between you and your parents	Suspension/expulsion from college or university
Suspension from school	Have become involved with drugs
Failing end of year exams	Had a major financial crisis
Getting pregnant or fathering a pregnancy	Break up of a steady relationship
Being responsible for a road accident	Failed end of year exams
Being told to break up with a boy/girl friend	Start of new problem between you and your parents
Being invited by a friend to break the law	Been sacked from a job
Breaking up with a boy/girl friend	Been invited by a friend to break the law
	Have failed to achieve something you really want

APPENDIX 1. Continued

	Times 1 and 2	Time 3
Independent stressful life events		
	Death of a close friend	Death of a close friend
	Being hospitalized for illness or injury	Been in hospital with a serious illness or injury
	Major increase in your parents' income	A parent hospitalized for a serious illness or injury
	Loss of a job by your father or mother	Death of a second degree relative (e.g. grandparent)
	Hospitalisation of a brother or sister	A sibling hospitalised for a serious illness or injury
	Remarriage of a parent to a stepparent	Had something valuable lost or stolen
	Hospitalisation of a parent	Death of a sibling
	Death of a grandparent	Death of a parent
	Marital separation of your parents	
	Divorce of your parents	
	The death of a brother or sister	
	The death of a parent	

Note: Participants were asked about stressful life events that had occurred in the past year at times 1 and 2 using a 24-item version of the Life Event Scale for Adolescents (LES-A),[32] and at time 3 (participants over 18 years), using the List of Threatening Experiences (LTE: 33), supplemented with nine items from the LES-A.[32]

APPENDIX 2. Number of participants, mean anxiety sensitivity score, and standard deviations across levels of the genotype and stressful life events

		5HTTLPR Genotype					
		L'L'		S'L'		S'S'	
		N	AnxS Mean (SD)	N	AnxS Mean (SD)	N	AnxS Mean (SD)
Self-rated stressful life events							
Dependent							
Time 1	0	115	28.02 (5.89)	206	27.80 (5.89)	94	28.36 (5.21)
	1	67	30.06 (5.32)	150	28.45 (5.03)	72	28.78 (5.18)
	≥ 2	80	30.87 (5.50)	178	30.39 (5.71)	91	30.01 (5.55)
Time 2	0	61	24.91 (5.33)	144	24.20 (5.41)	57	25.86 (4.99)
	1	53	25.40 (6.49)	114	25.62 (5.15)	45	25.05 (5.40)
	≥ 2	67	27.01 (6.38)	109	29.00 (6.41)	61	26.93 (5.50)
Time 3	0	13.81 (7.46)	86	178	13.29 (8.46)	89	13.81 (7.46)
	1	63	16.12 (7.25)	109	15.66 (8.99)	52	16.12 (7.25)
	≥ 2	57	15.63 (8.19)	142	18.18 (9.58)	60	15.63 (8.19)
Independent							
Time 1	0	165	28.90 (5.66)	317	28.27 (5.02)	155	28.44 (5.07)
	1	50	29.22 (5.54)	133	29.45 (5.56)	63	29.31 (5.19)
	≥ 2	47	31.43 (5.97)	84	30.06 (5.56)	39	31.12 (6.25)
Time 2	0	94	25.47 (6.32)	216	25.84 (5.82)	100	25.67 (5.43)
	1	56	25.20 (4.60)	97	25.35 (5.35)	40	26.48 (5.34)
	≥ 2	31	28.06 (7.46)	54	27.43 (7.06)	23	26.87 (4.87)
Time 3	0	118	16.09 (9.59)	248	15.15 (9.04)	105	13.71 (7.39)
	1	63	18.25 (10.79)	129	15.55 (8.69)	72	15.74 (7.81)
	≥ 2	25	16.34 (10.80)	52	17.13 (11.05)	24	18 (7.54)
Parent-rated stressful life events							
Dependent							
Time 2	0	88	25.72 (6.21)	199	25.53 (5.73)	75	26.36 (5.26)
	1	60	24.79 (5.06)	90	26.36 (6.16)	46	26.18 (5.35)
	≥ 2	20	27.20 (6.83)	53	26.67 (5.83)	31	25 (3.85)
Independent							
Time 2	0	94	24.95 (5.87)	213	25.93 (5.72)	101	26.12 (4.71)
	1	53	25.45 (5.97)	90	25.33 (5.52)	32	25.69 (5.64)
	≥ 2	21	29.29 (5.77)	40	26.88 (7.05)	19	25.37 (5.10)

Note: AnxS Mean, Anxiety sensitivity mean score; L'L', long–long genotype; L'S', long–short genotype; S'S', short–short genotype; *SD*, standard deviation.

Depression and Anxiety

APPENDIX 3. Main effect of *5HTTLPR*, and gene–environment interactions on anxiety sensitivity and change in anxiety sensitivity over time

		Anxiety sensitivity			Change in anxiety sensitivity		
					Time 2	Time 3	Time 3
					(Controlling for time 1 anxiety sensitivity)		(Controlling for time 2 anxiety sensitivity)
		Time 1	Time 2	Time 3			
Main effect of *5HTTLPR*		$\beta = -.02$	$\beta = .02$	$\beta = -.09$	$\beta = -.03$	$\beta = -.07$	$\beta = -.08$
Stressful life events*5HTTLPR							
		Self-rated stressful life events					
Dependent*5HTTLPR	Time 1	$\beta = -.05$	$\beta = -.03$	$\beta = .02$	-	-	-
	Time 2	-	$\beta = -.04$	$\beta = -.01$	$\beta = -.07$	$\beta = .00$	$\beta = .03$
	Time 3	-	-	$\beta = -.13$	-	$\beta = .02$	$\beta = .09$
Independent*5HTTLPR	Time 1	$\beta = .00$	$\beta = .01$	$\beta = .06$	-	-	-
	Time 2	-	$\beta = -.04$	$\beta = -.06$	$\beta = .04$	$\beta = .00$	$\beta = .01$
	Time 3	-	-	$\beta = -.02$	-	$\beta = .10$	$\beta = .09$
		Parent-rated stressful life events					
Dependent*5HTTLPR	Time 2	-	$\beta = -.09$	$\beta = .06$	$\beta = .10$	$\beta = -.06$	$\beta = -.01$
Independent*5HTTLPR		-	$\beta = -.17^{*}$	$\beta = -.06$	$\beta = -.11$	$\beta = -.04$	$\beta = -.03$

Note: [*]$P < .05$.

REFERENCES

1. Taylor S. Anxiety sensitivity: theoretical perspectives and recent findings. Behav Res Ther 1995;33:243–258.
2. Taylor S, Koch WJ, McNally RJ. How does anxiety sensitivity vary across the anxiety disorders? J Anxiety Disord 1992;6:249–259.
3. Benitez CI, Shea MT, Raffa S, et al. Anxiety sensitivity as a predictor of the clinical course of panic disorder: a 1-year follow-up study. Depress Anxiety 2009;26:335–342.
4. Schmidt NB, Lerew DR, Jackson RJ. The role of anxiety sensitivity in the pathogenesis of panic: prospective evaluation of spontaneous panic attacks during acute stress. J Abnorm Psychol 1997;106:355–364.
5. Grant DM, Beck JG, Davila J. Does anxiety sensitivity predict symptoms of panic, depression, and social anxiety? Behav Res Ther 2007;45:2247–2255.
6. Muris P, Schmidt H, Merckelbach H, Schouten E. Anxiety sensitivity in adolescents: factor structure and relationships to trait anxiety and symptoms of anxiety disorders and depression. Behav Res Ther 2001;39:89–100.
7. Reiss S, Havercamp S. The sensitivity theory of motivation: implications for psychopathology. Behav Res Ther 1996;34:621–632.
8. Schmidt NB, Lerew DR, Joiner TE, Jr. Prospective evaluation of the etiology of anxiety sensitivity: test of a scar model. Behav Res Ther 2000;38(11):1083–1095.
9. Zavos HMS, Gregory AM, Eley TC. Longitudinal genetic analysis of anxiety sensitivity. Dev Psychol 2012;48:204–212.
10. McLaughlin KA, Hatzenbuehler ML. Stressful life events, anxiety sensitivity, and internalizing symptoms in adolescents. J Abnorm Psychol 2009;118:659–669.
11. Stein MB, Jang KL, Livesley WJ. Heritability of anxiety sensitivity: a twin study. Am J Psychiatry 1999;156:246–251.
12. Eley TC, Gregory AM, Clark DM, Ehlers A. Feeling anxious: a twin study of panic/somatic ratings, anxiety sensitivity and heartbeat perception in children. J Child Psychol Psychiatry 2007;48:1184–1191.
13. Zavos HM, Rijsdijk FV, Gregory AM, Eley TC. Genetic influences on the cognitive biases associated with anxiety and depression symptoms in adolescents. J Affect Disord 2010;124:45–53.
14. Levinson DF. The genetics of depression: a review. Biol Psychiatry 2006;60:84–92
15. Lesch KP, Bengel D, Heils A, et al. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science 1996;274:1527–1531
16. Katsuragi S, Kunugi H, Sano A, et al. Association between serotonin transporter gene polymorphism and anxiety-related traits. Biol Psychiatry 1999;45:368–370.
17. Jorm AF, Prior M, Sanson A, Smart D, Zhang Y, Easteal S. Association of a functional polymorphism of the serotonin transporter gene with anxiety-related temperament and behavior problems in children: a longitudinal study from infancy to the mid-teens. Mol Psychiatry 2000;5(5):542–547.
18. Jorm AF, Henderson AS, Jacomb PA, Christensen H, Korten AE, Rodgers B, et al. An association study of a functional polymorphism of the serotonin transporter gene with personality and psychiatric symptoms. Mol Psychiatry 1998;3(5):449–451.
19. Uher R, McGuffin P. The moderation by the serotonin transporter gene of environmental adversity in the aetiology of mental illness: review and methodological analysis. Mol Psychiatry 2008;13:131–146.
20. Caspi A, Sugden K, Moffitt TE, et al. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science 2003;301:386–389.
21. Risch N, Herrell R, Lehner T, et al. Interaction between the serotonin transporter gene (5-HTTLPR), stressful life events, and risk of depression: a meta-analysis. Jama 2009;301:2462–2471.
22. Karg K, Burmeister M, Shedden K, Sen S. The serotonin transporter promoter variant (5-HTTLPR), stress, and depression meta-analysis revisited: evidence of genetic moderation. Arch Gen Psychiatry 2010;68:444–454.
23. Stein MB, Schork NJ, Gelernter J. Gene-by-environment (serotonin transporter and childhood maltreatment) interaction for anxiety sensitivity, an intermediate phenotype for anxiety disorders. Neuropsychopharmacology 2008;33:312–319.
24. Klauke B, Deckert J, Reif A, et al. Serotonin transporter gene and childhood trauma - a G × E effect on anxiety sensitivity. Depress Anxiety 2011;28:1048–1057.

25. Dahl RE, Gunnar MR. Heightened stress responsiveness and emotional reactivity during pubertal maturation: implications for psychopathology. Dev Psychopathol 2009;21(1):1–6.

26. Lau JY, Rijsdijk F, Eley TC. I think, therefore I am: a twin study of attributional style in adolescents. J Child Psychol Psychiatry 2006;47:696–703.

27. Liang H, Eley TC. A monozygotic twin differences study of nonshared environmental influence on adolescent depressive symptoms. Child Dev 2005;76:1247–1260.

28. Silverman WK, Fleisig W, Rabian B, Peterson RA. Childhood anxiety sensitivity index. J Clin Child Psychol 1991;20:162–168.

29. Reiss S, Peterson RA, Gursky DM, McNally RJ. Anxiety sensitivity, anxiety frequency and the prediction of fearfulness. Behav Res Ther 1986;24:1–8.

30. Peterson RA, Reiss S. Anxiety Sensitivity Index manual, International Diagnostics Systems. Palos Heights, IL: IDS Publishing 1987.

31. Cox BJ, Parker JD, Swinson RP. Anxiety sensitivity: confirmatory evidence for a multidimensional construct. Behav Res Ther 1996;34:591–598.

32. Coddington BF. Measuring the stressfulness of a child's environment. In: Humphrey JH, editor. Stress in Childhood. New York: AMS Press Inc.; 1984:97–126

33. Brugha T, Bebbington P, Tennant C, Hurry J. The list of threatening experiences: a subset of 12 life event categories with considerable long-term contextual threat. Psychol Med 1985;15:189–194.

34. Brown GW, Harris TO. Social Origins of Depression: A Study of Psychiatric Disorder in Women. London: Tavistock; 1978.

35. Silberg J, Rutter M, Neale M, Eaves L. Genetic moderation of environmental risk for depression and anxiety in adolescent girls. Br J Psychiatry 2001;179:116–121.

36. Hu X, Oroszi G, Chun J, Smith TL, Goldman D, Schuckit MA. An expanded evaluation of the relationship of four alleles to the level of response to alcohol and the alcoholism risk. Alcohol Clin Exp Res 2005;29:8–16.

37. Wendland JR, Martin BJ, Kruse MR, Lesch KP, Murphy DL. Simultaneous genotyping of four functional loci of human SLC6A4, with a reappraisal of 5-HTTLPR and rs25531. Mol Psychiatry 2006;11(3):224–226.

38. Parsey RV, Hastings RS, Oquendo MA, et al. Lower serotonin transporter binding potential in the human brain during major depressive episodes. Am J Psychiatry 2006;163:52–58.

39. Stata StataCorp; College Station, TX: 2007. Stata Statistical Software, Release 10.

40. Rogers WH. Regression standard errors in clustered samples. Stata Tech Bull 1993;13:19–23.

41. Williams RL. A note on robust variance estimation for cluster-correlated data. Biometrics 2000;56(2):645–646.

42. Jaffee SR, Price TS. Gene-environment correlations: a review of the evidence and implications for prevention of mental illness. Mol Psychiatry 2007;12(5):432–442.

43. Schmidt NB, Lerew DR, Jackson RJ. Prospective evaluation of anxiety sensitivity in the pathogenesis of panic: replication and extension. J Abnorm Psychol 1999;108:532–537.

44. Nolen-Hoeksema S. The role of rumination in depressive disorders and mixed anxiety/depressive symptoms. J Abnorm Psychol 2000;109:504–511.

45. Watkins ER. Constructive and unconstructive repetitive thought. Psychol Bull 2008;134:163–206.

46. Rutter M. Gene-environment interplay. Depress Anxiety 2010;27(1):1–4.

47. Eley TC, Sugden K, Corsico A et al. Gene-environment interaction analysis of serotonin system markers with adolescent depression. Mol Psychiatry 2004;9:908–915.

48. Dunn EC, Uddin M, Subramanian SV, Smoller JW, Galea S, Koenen KC. Research review: gene-environment interaction research in youth depression - a systematic review with recommendations for future research. J Child Psychol Psychiatry 2011;52(12):1223–1238.