Universiteit Utrecht

The influence of early life stress and the 5-HTTLPR polymorphism on stress responsiveness: a gene x environment approach

Master's Thesis Clinical and Health Psychology

Author: A.C. Sollie, MSc (3056260)

Supervision: Dr. E.R. Bossema

Prof. dr. L.J.P. van Doornen

Utrecht University

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Abstract

The current study focuses on nature x nurture interactions on individual differences in stress responsiveness. Stress plays a role in many psychological disorders giving rise to the need to map environmental and genetic factors contributing to individual differences in stress responsiveness. The effects of both early life stress, an environmental factor, and the 5-HTTLPR polymorphism, a genetic factor, on psychological and physiological stress responsiveness were examined using the TSST-G to induce stress. Although no main effects for any of the variables were found, the results support a possible inverse relation between early life stress and a psychological stress measure. It further shows evidence of possible differences in this relationship between S- and non-S-carriers of the 5-HTTLPR polymorphism.

Introduction

Heterogeneity in individual stress responsiveness has been ascribed to differences in genetic background (nature) as well as environmental factors (nurture) (Homberg, 2011). It has become clear that these individual differences cannot easily be attributed to either one of these. Rather genes and environmental factors interact, resulting in differences in stress responsiveness. This process of interaction between nature and nurture is applicable to psychiatric disorders, such as depression and anxiety disorders (Akkerman, Kaasik, Kiive, Nordquist, Oreland & Harro, 2011; Homberg, 2011). Experiencing adversity can lead to increased risk of developing depression, but the way in which adversity is capable of eliciting stress plays a major role in predicting negative outcomes (Ellis, Boyce, Belsky, Bakermans-Kranenburg & Van Ijzendoorn, 2011).

Stressful environmental experiences are known to elicit and exacerbate mental disorders like depression, bipolar disorder and schizophrenia (Homberg, 2011; Steen et al., 2011). Gaining insight in the factors contributing to differences in stress responsiveness is not only of theoretical importance but also of practical importance because it will help map genetic and environmental risk factors contributing to psychiatric disorders. Caspi et al. (2003) show the development of depression as an outcome of environmental stressors to be moderated by a polymorphism in the serotonin transporter linked polymorphic region (5-HTTLPR). Although the body of research on this subject is growing, there are questions that remain to be answered in the G x E field of 5-HTTLPR. Caspi et al. (2003) found the L-allele of the polymorphism to be associated with lower rates of depression following past stressors as opposed to the S-allele. Although these findings were replicated in other studies (Aguilera, 2009; Taylor et al., 2006), there are studies reporting no differences between the two alleles (Laucht et al., 2009; Wichers et al., 2008) and studies reporting an opposite effect (Zhang, Yang & Chan, 2009; Chorbov et al., 2007). Therefore, this study aimed to investigate the contribution of early life stress and the 5-HTTLPR polymorphism on psychological and physiological stress responsiveness in healthy human subjects. This will be done by answering the following question: What is the contribution of early life stress and the 5-HTTLPR polymorphism on psychological and physiological stress responsiveness, and do these variables show interaction?

To answer this question first the processes of psychological and physiological stress are described, after which these are discussed in light of the effects of early life stress. Next the influence of the 5-HTTLPR polymorphism in relation to psychological and physiological

stress responsiveness is discussed. Concluding in an integration of the theory in hypothesised relations between the variables will be given.

Stress responsiveness

A stressor is a stimulus or situation which may induce a physiological and psychological stress response. Whether a stressor evokes a stress response depends on its characteristics, on the individual's appraisal of the situation (primary appraisal) and on his / her perceived coping abilities (secondary appraisal) (Kaptein & Weinman, 2004). The psychological stress response may reflect feelings of anxiety, tension and insecurity.

Physiologically the body's initial response to stress is inhibition of the parasympathetic nervous system (PNS). The PNS promotes attention and relaxation by countering sympathetic nervous system (SNS) activity, thus its inhibition leads to promoting functions of the SNS which results in heightened levels of arousal (Del Giudice, Ellis & Shirtcliff, 2011). Continued promotion of SNS activity, due to persistence of the stressor, results in the fight or flight response which is under control of the Locus Coeruleus (LC) (Del Giudice et al., 2011). The LC secretes norepiniphrine, stimulating visceral organs and activating the adrenal medulla trough hormonal pathways (Bailey, Engler, Hunzeker & Sheridan, 2003). In reaction the adrenal medulla releases epinephrine and in lesser amounts norephinephrine (Elam, Thorén & Svensson, 1985). This results in increased heart rate, rate of respiration, glucose release in the bloodstream, blood supply to muscles and suppression of vegetative functions (Del Giudice et al., 2011; Luecken & Lemery, 2004; Perry, Pollard, Blakley, Baker & Vigilante, 1995).

A delayed physiological response to stress consists of cortisol secretion by the hypothalamic-pituitary-adrenal (HPA) axis (Daban, Vieta, Macking & Young, 2005). By increasing cortisol levels the HPA-axis facilitates energy release, increased alertness and vigilance, and memory sensitization (Del Giudice et al., 2011). Cortisol also inhibits the fight or flight response thus facilitating recovery and preventing adverse effects caused by long periods of high arousal (Del Giudice et al., 2011; Loman & Gunnar, 2010; Jansen, Gispen-de Wied, Gademan, De Jonge, Van der Linden & Kahn, 1998). To prevent overstimulation of the HPA-axis it is under control of a negative feedback system in which cortisol returns to the pituitary and hypothalamus to inhibit additional secretion of cortisol (Daban et al., 2005).

The amygdala plays a crucial role in the regulation of the components of the physical stress response system (Del Giudice et al., 2011) in that it is responsible for initiating release

of stress hormones, activating the SNS, initiating fight or flight behaviour and processing emotional reactions (Brewin, 2003).

Experiencing stress is a complex process; individual differences in previous experience and genetic predisposition could account for variations in any of the involved psychological or physical processes, thus facilitating differences in stress responsiveness.

Early life stress

Individual differences in stress responsiveness are in part related to environmental factors (Kaptein & Weinman, 2004). A major contribution is made by experiences during childhood, especially early life stress, defined as reactions to stressors experienced in pre-puberty (Loman & Gunnar, 2010). Research on the effects of early life stress comes with methodological difficulties, because an experimental design would be unethical it relies on naturally occurring events and uses retrospective reports. Despite these difficulties a growing body of research on the subject is available.

The physiological effects of early life stress on stress responsiveness later in life have mainly been investigated in terms of cortisol secretion following a stress task and show a blunted but enduring cortisol response to stressors as a result (Lovallo et al., 2012; Claessens et al., 2011; Loman & Gunnar, 2010; Klaassens et al., 2009; Carpenter et al., 2007). Other physiological effects of early life stress on the brain are described as the development of a bias in the developing stress response system leading to greater defensive reactions (fight or flight) elicited by relatively minor stressors (Perry, Pollard, Blakley & Vigilante, 1995). Evidence for these changes in the SNS response due to early life stress was found as an increased hear rate following a stressor in 10-12-year-olds (Gunnar, Frenn, Wewerka & Van Ryzin, 2009) as well as adult women (Heim et al., 2000).

In a recent study Lovallo, Fagar, Sorocco, Cohoon & Vincent (2012) were not able to replicate these results; they tested healthy participants of both sexes and used a higher number of participants in their study than in previous studies on the subject. Their results showed a similar effect for heart rate as for cortisol, both declined with an increase in the number of stressful incidents experienced during childhood. Psychological levels of distress were also measured showing no differences between groups.

The effects of early life stress on stress responsiveness are mainly explained as a result of heightened sensitivity to stress due to adverse childhood experiences that teach the child the world is not a safe place (Smeets, 2010). This perspective however fails to explain the blunted cortisol response, which would be expected to accompany a less intense stress

response as found by Lovallo et al. (2012). Lovallo et al. (2012) showed the blunted cortisol and SNS response is not accompanied by a significant change in reported psychological stress; this leads them to believe early life stress could influence brain physiology but leaves psychological responsiveness intact.

In short it seems clear early life stress is an environmental factor contributing to individual differences in stress responsiveness. It leads to a blunted physiological response but seems to leave the psychological response unaffected.

5-HTTLPR

As a genetic factor affecting consequences of stressful life experiences and stress responsiveness recent research has focussed on the serotonin transporter gene-linked polymorphic region (5-HTTLPR). The serotonin transporter is involved in re-uptake of serotonin, thus affecting effectiveness of serotonin in the brain. The short or S-allele of the 5-HTTLPR is associated with lower transcription of the transporter as compared to the long or L-allele, and has been suggested to lead to less binding and re-uptake of serotonin. This makes S-allele carriers biologically more reactive to stress related stimuli (Akkerman et al., 2011).

The S-allele of 5-HTTLPR as opposed to the L-allele has been associated with higher stress responsiveness in that carriers seem to have more anxiety related personality characteristics (Lesch et al., 1996); greater amygdala activation was shown in a meta-analyses (Munafò, Brown & Hariri, 2008) which is evidence of greater emotional reaction to stressors (Lemogne et al., 2010); carriers showed stronger and longer lasting reactions to fearful stimuli (Akkerman et al., 2011); and higher levels of anxiety due to daily stressors (Klauke et al., 2011).

In addition to interacting with short term stress responsiveness the 5-HTTLPR also seems to affect long term consequences of stressful life events. In a longitudinal study Caspi at al. (2003) found carriers of the L-allele less likely to develop depression after stressful life events. Replication of this study has led to mixed results (Caspi, Hariri, Holmes, Uher & Moffitt, 2010). Some studies finding the S-allele to be associated with higher likeliness of depression after early life stress (Aguilera, 2009; Taylor et al., 2006) some finding there to be no difference between S and L-allele carriers (Laucht et al., 2009; Wichers et al., 2008) and some finding L-allele carriers to be at higher risk of depression after early life stress (Zhang, Yang & Chan, 2009; Chorbov et al., 2007). This makes it difficult to make predictions about a general protective effect of 5-HTTLPR on the effects of early life stress and although the S-

allele is associated with higher stress responsiveness, it is not yet clear if S-carriers are more likely to be diagnosed with depression following life stressors than L-carriers (Caspi et al., 2010).

A direct analogy between the effects of 5-HTTLPR on developing psychiatric disorders and the effects on stress responsiveness cannot be drawn. However, because in both cases an effect of adverse experience is present and because the polymorphism is linked to biological mechanisms involved in stress responsiveness it is probable it also affects the effects early life stress has on stress responsiveness. Although the mixed findings of studies on the effects of both alleles on psychiatric disorders following adverse events make it difficult to make a clear cut prediction most evidence points in the direction of a protective effect of the L-allele of the 5-HTTLPR (Caspi et al., 2010).

Current study

The aim of the current study was to examine the influence of early life stress as an environmental factor and of the 5-HTTLPR polymorphism as a genetic factor on the stress responsiveness following a stress task in healthy male subjects. Subjective stress responsiveness was measured using visual analogue scales. Autonomic stress responsiveness was measured as a rise in mean heart rate. It was hypothesized that more early life stress would lead to a lower physiological stress response but would not affect the psychological stress response. It was further hypothesized that possession of the heterozygous or homozygous S-allele of the 5-HTTLPR polymorphism as compared to a homozygous L-allele would show a higher physiological stress responsiveness but no difference with respect to the psychological stress responsiveness. Moreover, it was expected that the combination of high early life stress and possession of an S-allele of the 5-HTTLPR polymorphism would lead to an interaction effect leading to the lowest physiological stress responsiveness compared to low early life stress and not possessing an S-allele, no effect was expected for psychological stress responsiveness.

Methods

Participants

The current study was carried out among participants of an earlier study which focused on the effects of cannabis use on psychotic symptoms. Participants included in the current study were non-users or low users of cannabis. For the previous study participants donated blood for genetic profiling. Participants in the current study were recruited by telephone. The phone call included a structured interview to examine study eligibility by excluding anyone who used medication, met criteria for a psychological disorder or smoked on a daily basis.

A total of 31 healthy male participants were included. Participants were between the ages of 18 and 29, with a mean age of 23.2 and a standard deviation of 2.5. Because Body mass index (BMI) can influence heart rate (Shekharappa, Smilee, Mallikarjuna, Vedavathi & Jayarajan, 2011) BMI scores were calculated for all participants these ranged from 18 to 29, with a mean of 22.3 and a standard deviation of 2.7. Four people were overweight (BMI > 25) but on average participants were of healthy weight. These scores were used for correction during analyses.

Stress induction

Stress was elicited using the Trier Social Stress Test for Groups (TSST-G) (Von Dawans, Kirschbaum & Heinrichs, 2010) this is a standardized motivated performance task that combines socio-evaluative threat and uncontrollability in a group format (Von Dawans et al., 2010). Contrary to the original TSST-G, which is designed to stress groups of 6 people, in the current study 3 to 4 participants participated in the test which consists of a 5 minute speech task (a mock job interview) and a 5-minute arithmetic task (counting back in steps of 17). The stress test lasted for about 16 minutes.

Instruments

Early life stress was measured using the Childhood Trauma Questionnaire Short Form (CTQ) developed by Bernstein et al. (2003). This 24-item version was derived from the original 70-item Childhood Trauma Questionnaire (Bernstein & Fink, 1998; Bernstein et al., 1994). The Dutch translation used in this study has one less item on the sexual abuse scale due to translation issues from the original English version (Thombs, Bernstein, Lobbestael & Arntz, 2009). The questionnaire consists of 5 scales: emotional abuse (5 items, for example “People in my family called me things like ‘stupid,’ ‘lazy,’ or ‘ugly’”; Cronbach’s $\alpha = .64$ in the

current study), physical abuse (5 items, for example “People in my family hit me so hard it left me with bruises or marks”; α=.44), sexual abuse (4 items, for example “Someone tried to make me do sexual things or watch sexual things”; α could not be calculated due to low reports), emotional neglect (5 items, for example the reverse of “I felt loved”; α = .86) and physical neglect (5 items, for example “I didn’t have enough to eat”; α = .08). Answers could be given on a 5 point scale ranging from 1 (“never true”) to 5 (“very often true”). The total score range of the CTQ was 24 to 120 (α = .79, based on 18 items with variance).

State anxiety, reflecting the psychological stress responsiveness, was measured with various Visual Analogue Scales (VAS’s; Lesage & Berjot, 2011). The VAS consisted of 5 statements on feelings and symptoms of anxiety (for example: “I feel stressed”). The participants indicated on a 10 cm line, ranging from “not at all” to “very much”, their agreement with each statement. The statements were completed 10 minutes before (α = .50), about 5 minutes after the start (α = .73), and 35 minutes after the end of the TSST-G (α = .65). To compute total scores for the three VAS measurements the 10 cm lines were measured in centimetres and scores for the 5 questions were added up. The difference between the three VAS scores (VAS2-1, VAS3-1) was also computed and taken as a measure of psychological stress responsiveness comparing stress during to before and after the task.

Physiological stress was operationalized as heart rate, reflecting SNS activity. Heart rate was continuously recorded using the Suunto t6d and expressed as beats per minute (bpm). Three 10-minute intervals were used for analyses: one starting 41 minutes prior to the TSST-G, one starting 2 minutes after the start of the test and one starting 62 minutes after the start of the test. During the intervals before and after the TSST-G participants were sitting down, reading light literature in a quiet room together with at least two other participants. The choice for 10 minute intervals was made because this was the longest time of overlap during the TSST-G available over all subjects. This was due to differences in the protocol length attributable to unforeseen situations caused by both the participants and the researchers. The intervals were further chosen on the grounds of minimal distortion of heart rate data in all participants, caused by heart rate recorder failure.

5-HTTLPR

Genotype data for subjects was generated on three different array platforms: Illumina Human Omni Express, Illumina Human610-Quad Beadchip and Illumina Human Hap550. Quality control was done as described by Wray et al. (2009). Differentiation between 5-HTTLPR S and L-alleles was made using proxy single nucleotide polymorphisms consisting of the TA

haplotype of rs2129785 and rs11867581 that tag the S-allele (r^2=.78) (Vinkhuyzen et al., 2011). Genotypic data were classified in two groups, L/L homozygotes or non-S-allele carriers (*n = 4*) and S-allele carriers (*n* = 19).

Procedure

To control for the effects of circadian cortisol levels (not examined in this study), all sessions ran between 13.00h and 17.00h. Participants were asked not to eat, brush their teeth or chew bubblegum 2 hours prior to participation. They were further asked to abstain from caffeine containing drinks 4 hours prior to participation, heavy physical activity 12 hours prior to participation and alcohol 24 hours prior to participation to eliminate differences in stress reactivity during the study due to any of these factors. After arrival participants were geared up with a heartbeat sensor and asked to quietly read in a waiting room together with at least two other participants to ensure a steady resting baseline of heart rate. During this time participants filled in the VAS, STAI-S and CTQ. After 45 minutes participants were given 5 minutes time to prepare for the mock job interview which was part of the TSST-G. Between the interview and the arithmetic task of the TSST-G participants filled in a second VAS. After the test participants again took seat in the waiting room, completing a third VAS and a second STAI-S, after which they returned to reading. The total duration of the procedure was 3 hours, after which the heart rate recorders were removed and the data was transferred to a computer.

Statistical analyses

Analyses were done using SPSS 15.0 for Windows. First it was checked whether the TSST-G did cause a psychological and physiological stress response. This was done by comparing the VAS scores before, during and after the test using paired samples *t*-tests. Then the mean differences were calculated for the VAS and heart rate scores for the scores before and during, and during and after the TSST-G. These difference scores together with the CTQ total score and the CTQ subscales scores were then checked for normality of their distribution with the Kolmogorov-Smirnov test. A non-normal distribution was only found for the CTQ subscales emotional abuse, sexual abuse and physical neglect, due to relatively low scores. Since the internal consistencies (Cronbach's α) for most of these subscales were also low, subsequent analyses of the CTQ were only performed using the total score.

Univariate and multiple linear regression analysis were used to examine the independent and combined influence of early life stress (CTQ total score) and of the 5-HTTLPR polymorphism on the stress responsiveness of the participants, as reflected by the

two VAS difference scores and the two heart rate difference scores. Corrections for BMI were carried out where possible. Outcomes with $p < .05$ were considered statistically significant.

Results

Missing data

Heart rate data were missing for 13 participants due to recorder failure, leaving data of 18 participants for the analyses with heart rate. In addition, data on 5-HTTLPR were missing for 8 participants, leaving 23 participants for the analyses with genotype. Data on either heart rate or genotype were missing for 19 participants, leaving data of 12 participants for the analyses with both heart rate and 5-HTTLPR.

Descriptives

Table 1 shows the mean scores, standard deviations and score range with respect to the measures of early life stress (CTQ) and the psychological (VAS and STAI-S) and physiological (heart rate) stress responses. The mean score on the total CTQ was low compared to the maximum possible score, indicating that participants reported relatively few traumatic events in their youth. Nevertheless, the majority of participants scored higher than 5 on at least one of the subscales: 16 (51.6%) reported emotional abuse, 6 (19.4%) reported physical abuse, 25 (80.6%) reported emotional neglect and 17 (54.8%) reported physical neglect.

Table 1. Means and Standard Deviations (SD) of all variables.

	n	Mean	SD
CTQ	31	31.2	6.0
VAS2-1	31	83.4	57.6
VAS2-3	31	102.5	54.2
HR2-1	18	22.9	12.2
HR2-3	18	23.6	12.4

CTQ = Childhood Trauma Questionnaire, VAS = Visual Analogue Scale, HR = Heart Rate.

Stress induction task

Participants had significantly higher VAS scores during the TSST-G (M = 164.6, SD = 71.4) than before the TSST-G (M = 81.2, SD = 50.7), $t(30) = -8.06$, $p < .01$, $r = .83$, and after the TSST-G (M = 62.1, SD = 51.5), $t(30) = -10.54$, $p < .001$, $r = .89$. The VAS scores after the TSST-G were significantly lower compared to the VAS scores before the TSST-G, $t(30) = 3.48$, $p < .01$. The heart rate during the TSST-G (M = 98.6, SD = 17.6) showed a significant increase compared to the heart rate before the test (M = 75.7, SD = 9.3) $t(17) = -7.99$, $p <$

.001, r = .89, and compared to after the test (M = 74.9, SD = 10.9) $t(17) = 8.10$, $p < .001$, r = .89. These findings indicate that the TSST-G indeed induced stress.

Main effects of early life stress and 5-HTTLPR on stress responsiveness

Table 2 shows the outcomes of the univariate linear regression analyses for each of the outcome measures. There were no significant main effects of the CTQ total score on the difference scores of the VAS and heart rate. In addition no significant main effects were found for 5-HTTLPR (comparing possession to no possession of the S-allele) on the difference scores of the VAS and heart rate either. This means there is no evidence for an independent influence of either early life stress or the 5-HTTLPR polymorphism on both psychological and physiological stress responsiveness.

Table 2. Results of univariate linear regressions with the main effects of CTQ scores and 5-HTTLPR on psychological and physiological stress measures.

	CTQ					5-HTTLPR				
	B	SE	β	R^2	P	B	SE	β	R^2	P
VAS2-1	2.85	1.71	.30	.09	.11	-33.50	29.22	-.24	.06	.27
VAS2-3	1.94	1.64	.21	.05	.25	-.38	28.62	-.00	.00	.99
HR2-1	.28	.45	.16	.02	.51	-6.32	10.91	-.18	.03	.58
HR2-3	.52	.44	.28	.08	.26	-11.76	10.03	-.35	.12	.27

CTQ = Childhood Trauma Questionnaire, VAS = Visual Analogue Scale, HR = Heart Rate.

Interaction effects of early life stress and 5-HTTLPR on stress responsiveness

Table 3 shows the outcomes of the multiple regression analysis, including both the CTQ total score and 5-HTTLPR as well as the interaction between these two variables for each of the outcome measures. A significant effect was found relating to the VAS change from before to during the TSST-G: participants without the S-allele and low CTQ scores reported the highest increase in VAS scores during the TSST-G as compared to before (p = .012) (Figure 1). A second significant interaction was found for the VAS change from after to during TSST-G (p=.016) this also shows the non-S-allele, low CTQ score participants to have the largest difference between baseline and TSST-G (Figure2).

Table 3. Results of multiple regression on the interaction between CTQ and Genotype in predicting psychological and physiological stress measures (n = 23).

		B	SE	β	R^2
VAS2-1					
1.	BMI	.48	4.13	.03	.00
2.	BMI	-.52	3.97	-.03	.20
	CTQ	3.27	1.80	.39	

		5-HTTLPR	-21.80	29.16	-.16	
	3.	BMI	-1.09	3.40	-.06	.45
		CTQ	-1.52	2.29	-.18	
		5-HTTLPR	-30.51	25.17	-.22	
		CTQx5-HTTLPR	51.66	18.37	.75*	
VAS2-3						
	1.	BMI	.21	3.93	.01	.00
	2.	BMI	-1.09	3.90	-.06	.15
		CTQ	3.17	1.76	.40	
		5-HTTLPR	11.29	28.64	.09	
	3.	BMI	-1.62	3.40	-.09	.39
		CTQ	-1.33	2.29	-.17	
		5-HTTLPR	3.09	25.16	.02	
		CTQx5-HTTLPR	48.60	18.37	.74*	
HR2-1						
	1.	BMI	-1.31	1.64	-.25	.06
	2.	BMI	-1.17	1.84	-.22	.09
		CTQ	.16	.72	.09	
		5-HTTLPR	-4.27	13.45	-.12	
	3.	BMI	-1.38	1.92	-.26	.15
		CTQ	.81	1.17	.44	
		5-HTTLPR	2.04	16.45	.06	
		CTQx5-HTTLPR	-6.46	9.04	-.41	
HR2-3						
	1.	BMI	-1.58	1.55	-.31	.09
	2.	BMI	-1.26	1.63	-.24	-.05
		CTQ	.40	.63	.23	
		5-HTTLPR	-7.42	11.88	-.22	
	3.	BMI	-1.50	1.66	-.29	-.07
		CTQ	-1.13	1.01	.64	
		5-HTTLPR	-.31	14.20	-.01	
		CTQx5-HTTLPR	-7.28	7.80	-.47	

*) $p < .05$ **) $p < .01$.

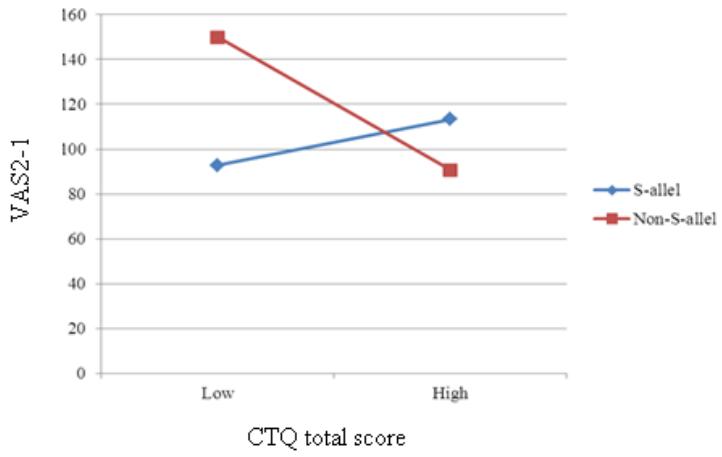

Figure 1. VAS change score from before to during the TSST-G predicted by the CTQ score and the 5-HTTLPR polymorphism (S-allele and non-S-allele).

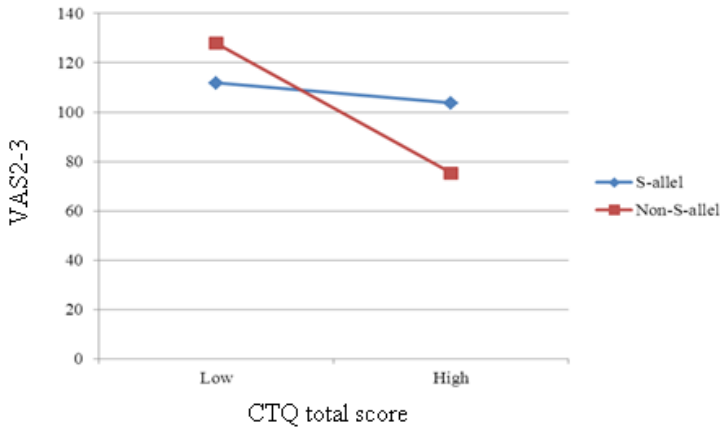

Figure 2. VAS change score form after to during the TSST-G predicted by the CTQ score and the 5-HTTLPR polymorphism (S-allele and non-S-allele).

Discussion

The aim of the current study was to examine the independent influence of early life stress as an environmental factor and of the 5-HTTLPR polymorphism as a genetic factor on the stress responsiveness following a stress task.

Early life stress and stress responsiveness

No evidence for the hypothesised link between early life stress and stress responsiveness, both psychological and physiological, could be found. This is not in agreement with some previous studies which found evidence linking early life stress to physiological stress measures (Gunnar, Frenn, Wewerka & Van Ryzin, 2009; Heim, et al., 2000). These studies however tested participants with more severe early life stress than participants in the current study. The results could therefore be evidence that the relation between early life stress and stress responsiveness does not exist in persons exposed to relatively low early life stress. It could be a threshold of early life stress has to be reached for it to affect stress responsiveness. Relatively mild early life stress also doesn't rule out a supportive early life environment, which, as Taylor (2006) describes, predicts less negative effects of current stressors. Further research is necessary to elucidate upon the mechanisms at work here.

5-HTTLPR and stress responsiveness

This study could not find evidence for the hypothesised link between the 5-HTTLPR polymorphism and both psychological and physiological stress responsiveness. It is hard to interpret these findings in the light of the findings of Akkerman et al. (2011) and Klauke et al. (2011) predicting a higher stress response to fearful stimuli and daily stressors respectively. But it does show the differences in stress responsiveness between the L- and S-allele carriers is not as strong as to show a significant difference with small group sizes as in the current study. The same is true for expectations on the ground of greater amygdala activation in S-allele carriers as described by Munafò, Brown & Hariri (2008). There might very well be a difference between L- and S-allele carriers of the 5-HTTLPR but it is not as profound as to rear its head in a small sample size study like the current.

5-HTTLPR x CTQ and stress responsiveness

No evidence could be found for the influence of both 5-HTTLPR and early life stress on the physiological stress responsiveness. Although the connection between the protective

properties of the 5-HTTLPR on the effect of early life stress is reported in previous research (Klauke et al., 2011; Caspi at al., 2003) no evidence to support this could be found in the current study. It could be this effect simply does not exist for hear rate. Another explanation for these findings could be the degree of early life stress in the participants of the current study. Research which found a protective effect of the polymorphism used participants who had been subjected to severe and sometimes debilitating early life stress. Participants in the current study were healthy and early life stress was relatively mild in all subjects. This difference is the most probable cause of the failure to find significant results. In summary the findings of the current study seem to indicate that no protective effect of 5-HTTLPR on an increase in physiological stress responsiveness following low levels of early life stress exists.

Contrary to what was hypothesised an effect was found for the psychological stress response. First the results for subjective stress during and before the TSST-G (figure 1) will be discussed. These results showed the highest difference for the low early life stress, non-S allele group, this was also the highest measured score. Least stress was expressed by the non-S-carriers with high early life stress. The results shows a decrease in reported stress combined with an increase in early life stress for non-S-carriers. This is an indication of an inverse connection between early life stress and stress responsiveness for non-S-carriers. It is as if a desensitisation towards stress following early life stress occurs in this group.

For the S-allele carrier group early life stress seems to lead to higher stress responsiveness in this condition; early life stress seems to sensitise S-carriers to stress. The scores for the low early life stress S-carriers are lower than scores for the low early life stress non-S-carriers, the S-allele does not seem to make people more vulnerable to psychological stress, in fact they seem more resilient.

The results of the subjective stress measures during and after the TSST-G (figure 2) show a similar relation between early life stress and stress responsiveness as the previous condition for non-S-carriers. Low early life stress non-S-carriers again have the highest score of all groups and there is an inverse connection between early life stress and stress responsiveness, high early life stress non-S-carriers having the lowest score of all groups. This is more evidence for a desensitisation process at work in non-S-carriers.

The S-carrier group in this condition also shows an inverse relation between early life stress and stress responsiveness, although the group difference is smaller than in the non-S-carrier group. As in the previous condition the S-allele seems to be accompanied by less psychological stress responsiveness.

In conclusion it seems non-S-carriers of the 5-HTTLPR have higher psychological stress responsiveness than S-carriers. This effect however seems to fade when early life stress is high, then psychological stress is lowest for the non-S-carriers. It could be non-S-carriers adapt to early life stress by means of lowering their psychological stress responsiveness. Evidence for this inverse relation between early life stress and psychological stress responsiveness was found for al groups in all conditions except for S-carrier difference scores before-during the TSST-G. Because S-carrier difference scores for psychological stress between during and before the TSST-G showed the opposite direction, these results are hard to interpret. The current study however does indicate a possible inverse connection between early life stress and psychological stress responsiveness, further research could focus on this connection in order to confirm it and elucidate upon the processes at work. Because a difference was found between S and non-S-carriers in this respect, the role of the 5-HTTLPR should also be taken into account in future research on this subject.

Limitations

The main limitation of this study was the small sample size, especially for heart rate and 5-HTTLPR. Another difficulty with the current study and doing research on genotypes in general is the uneven distribution of genes in the population. The majority of subjects in this study were heterozygotes for the 5-HTTLPR polymorphism, leaving only 4 subjects non-S-carriers.

A further point of attention is the generalizability of the results, because all participants were male. Women tend to react differently to stress, varying throughout the menstrual cycle phase and due to oral contraceptives (Krischbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999). What also makes generalization difficult is that all participants included in this study had previously taken part in an earlier study, so in fact a double selection took place; one for the previous study and one for this study.

Another limitation is the measurement of early life stress using the CTQ. The CTQ relies on retrospective self reports. The answers may therefore be subject to recall bias or participants could not have answered truthfully.

As Taylor (2006) describes there is a protective effect of recent positive experience on the reports of depressive symptoms in people with two S-alleles. It could be these protective effects also influence the effect of the S-allele on the stress response leading to recent positive experience to buffer for an increased stress response, for example by means of increased self-efficacy on the stress test. Although it cannot be stated our participants had more positive than

negative recent experiences, it seems likely participants with recent negative experiences declined participation in the current study or rescheduled their appointment for a test day. This might very well have influenced the results.

Conclusion

The main finding of this study is support for a possible inverse relation between early life stress and the degree of psychological stress during a stress test. The current study further shows evidence of possible differences in this relationship between S- and non-S-carriers of the 5-HTTLPR polymorphism.

While this study started out as an investigation of factors contributing to differences in interpersonal vulnerabilities it instead found evidence for factors contributing to resilience in the face of early life stress. In doing so it opens up new possibilities in mapping the environmental effects of early life stress and the influence of genotypic effects of 5-HTTLPR on psychological stress.

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