Reprinted from the German Journal of Psychiatry http://www.gjpsy.uni-goettingen.de ISSN 1433-1055

Differing Models of Association between Childhood Events, Recent Life Stressors, Psychological Resilience and Depression across Three Alleles of the Serotonin Transporter 5-HTTLPR

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Abstract

Background: Although some previous research has implicated the short form (ss) of the serotonin transporter 5-HTTLPR gene in the association between distal and proximal environmental stress and depression, over 38% of studies included in a recent meta-analysis failed to support that finding. Another variant of the 5-HTTLPT, the sl, has been relatively under-examined and may explain the inconsistency of the ss/ll dichotomy. In addition, a potential "buffer" variable between proximal and distal stress and depression – psychological resilience – may interact with the forms of the 5-HTTLPR. This study investigated the ways the three forms of the 5-HTTLPR interacted with distal and proximal stress, and psychological resilience, to predict depression.
Methods: A volunteer community sample of 65 female and 55 male volunteers completed background, childhood stress (Adverse Childhood Events-ACE), recent stress (RLS), depression (Zung Self-Rating Depression Scale-ZSDS) and resilience (Connors-Davidson Resilience Scale-CD-RISC) questionnaires, plus gave a sample for genotyping to determine presence of ss, sl, or ll variants of the 5-HTTLPR.
Results: Comparison of the regression equations for each 5-HTTLPR variant showed that the combination of ACE, RLS and CD-RISC significantly predicted ZSDS scores for the sl variant; ACE, CD-RISC (but not RLS) significantly predicted ZSDS for the ll variant; and none of these significantly predicted ZSDS for the ss variant.
Conclusions: Previous inconsistent findings regarding the differences in the stress-depression interaction for the ss and ll may be explained by the more complex interaction effects of the sl variant with distal and recent stressors and psychological resilience (German J Psychiatry 2013; 16(3): 103-111).

Keywords: genes, stress, depression, resilience

Received: 10.3.2013
Revised version: 30.7.13
Published: 15.8.2013

Introduction

The search for reliable ways of describing the influential factors underlying development of depression following stress has largely dealt with the interaction of environmental stressors and the serotonin transporter 5-HTTLPR gene (Caspi et al., 2010; Caspi et al., 2003; Grabe et al., 2011). In particular, that research has focussed upon the prevalence of depression among individuals who carry the short (*ss*) form of the 5-HTTLPR and who have experienced distal stressful life events (such as childhood adversity) as well as more recent proximal stressors (e.g., financial, relationship, and occupational difficulties). Karg and colleagues (2011) reported that there was "strong evidence" (p. 444) of the *ss* allele of the 5-HTTLPR being associated with an increased risk of individuals developing Major Depressive

Disorder (MDD) following distal (childhood) stressors, major medical conditions, and (less robust but still statistically significant) more recent (proximal) stressors. However, of the 54 studies that Karg et al. included in their meta-analysis, 15 reported no association between the *ss*, stress and depression and a further 6 reported that carriers of the long (*ll*) allele were more likely than *ss* carriers to become depressed following significant life stress (Karg et al., 2011, Table 1). Further, those studies which reported that *ll* carriers were more likely to become depressed after stress had sampled participants from quite different ages and populations, suggesting some degree of generalisability of the *ll*-stress-depression finding across culture and geography. These inconsistent previous findings suggest that comparison of the relationships between distal and proximal stressors and depression within each of the forms of the 5-HTTLPR might help explain the ways in which these genetic factors interact with environmental factors to increase the likelihood of depression occurring. As well as the *ss* and *ll* forms, there is a combined *sl* form of the 5-HTTLPR and that might also benefit from examination in this way.

In addition to these genetic and environmental 'causal' factors for depression, there are some alternative 'buffer' factors that have been reported as protecting individuals who suffer stress from developing depression. One of these is psychological resilience (Fredrickson et al., 2003), which refers to an individual's capacity to cope with stressors and to resist the harmful effects of future negative events (Luthar and Cicchetti, 2000), possibly by an active physiological process that reduces autonomic responses to stressors (Charney, 2004). As well as having been shown to intervene between the experience of traumatic events and the individual's later return to optimism in the face of such occurrences as old age (Jopp and Rott, 2006), terrorist attacks (Bonanno et al., 2007) and chronic pain (Karoly and Reuhlman, 2006), resilience has been shown to assist individuals overcome the experience of trauma during early childhood and to progress to normal and satisfying lives (Watt et al., 1995) and this has particular relevance for studies of the interaction between distal and proximal stressors, 5-HTTLPR and depression. Resilient behaviour has links with brain functions such as the plasticity of reward and fear circuits (Bergstrom et al., 2007; Feder et al., 2009) and there are at least 11 possible neurological mediators of the resilient response to stress (Charney, 2004). As a further indicator of the inconsistent findings regarding the various forms of the 5-HTTLPR, different studies have reported that: the *ss* form of the 5-HTTLPR was significantly linked with lower resilience (and potentially higher depression) compared to the *ll* (2009); the *ll* was significantly associated with lower resilience and higher depression than the *ss* (2011); and there was no significant association between either the *ss* or *ll* alleles of the 5-HTTLPR and resilience (2012). These inconsistencies leave the precise nature of the association between these forms of the 5-HTTLPR, resilience, stress and depression undefined.

Thus, although there has been considerable cohort research into the relationship between the 5-HTTLP alleles and depression and other mood disorders, the conclusions from those studies have often been contradictory. Alternately, there have been some studies on the biological effects of these genotypes which demonstrate that the *ss* allele results in less functional transporter (SERT) than the *ll* allele and (Heils et al., 1996; Lesch et al., 1996). Therefore, although there is some controversy over the dominance of the s or l allele in heterozygous subjects, it is likely that the *sl* falls somewhere between these two variants (Mössner et al., 2000). However, relatively few studies have closely examined the ways in which biological and psychological factors have interacted with the 5-HTTLPR, and none have been reported to date which included the *sl* form as well as the *ss* and *ll* forms of the 5-HTTLPR.

Finally, although most studies on the 5-HTTLPR have been conducted with MDD patients, some recent reports have focussed upon community samples to determine if effects generalise to non-MDD participants (e.g., Hovens et al., 2012), an approach which is directly relevant to the development of models of the associations between genetic, environmental and psychological factors in the wider population. Selecting community samples (instead of MDD patients) enables further investigation of the ways that these factors interact to produce depressive behaviour before the more severe forms of depression become evident.

Therefore, this study was designed to compare the relative ways in which the three forms of the 5-HTTLPR (*ss*, *ll*, *sl*) interacted with past and recent stressors and psychological resilience to predict depression across a wide range of clinical severity as well as for clinically significant depression. Although most previous research has followed a direct comparison model of the *ss* and *ll* forms of the 5-HTTLPR and depression, it was decided to examine each of those forms (plus the *sl* combination) separately in order to more clearly explicate each form's association with stress and depression rather than confound those individual 'causal' profiles by direct orthogonal analysis procedures.

Material and Methods

Participants: 65 females and 55 males volunteered for a study about "how you think about stress". They were aged between 18 and 69 years (M = 32.53yr, SD = 13.49 yr) and were recruited from the general population of a large regional city of about 22,000 people in New South Wales, Australia. To maximise generalisability to the population, no attempt was made to screen participants apart from ensuring they were at least 18 years of age.

Instruments

Background questionnaire: Age, gender and whether participants were currently taking antidepressant medication.

Depression: The Zung Self-Rating Depression Scale (ZSDS) (Zung, 1965) is a standardised paper and pencil test of depression that was developed on the basis of factor analytic studies of the syndrome of depression which underlie the DSM definition (APA, 2000). The ZSDS includes items for all of the current DSM-IV-TR criteria for Major Depressive Episode (MDE) (Zung, 1965), and has 20 items on which

respondents are asked to indicate the frequency of occurrence to them "during the last two weeks" by answering in one of four possible ways: "None or a little of the time", "Some of the time", "Good part of the time", or "Most or all of the time". Raw scores range from 20 to 80, with higher scores being indicative of more severe depression. The ZSDS has demonstrated split-half reliability of .81 (Zung, 1965), .79 (DeJonge and Baneke, 1989) and .94 (Gabrys and Peters, 1985). Internal consistency (alpha) has been reported as .88 for depressed patients and .93 for non-depressed patients (Schaefer et al., 1985), and as .84 for a previous Australian community sample (Sharpley and Rogers, 1985). The ZSDS has been shown to be superior to the MMPI Depression Scale and the Beck Depression Inventory for assessing depression in male psychiatric inpatients (Schaefer et al., 1985) and has sensitivity of 93% in predicting depression validated via clinical interview (Agrell and Dehlin, 1989). ZSDS raw scores of 40 or above indicate the presence of "clinically significant depression" (Zung, 1973, p. 335) and raw scores were used in this study.

Negative Childhood Events: The Adverse Childhood Events (ACE) questionnaire is a retrospective self-report inventory consisting of 30 statements relating to emotional, physical and sexual abuse as well as emotional and physical neglect which occurred during childhood. The ACE questionnaire was developed by the Centers for Disease Control and Prevention (CDC) and Kaiser Permanente in San Diego, using 17,000 participants (Felitti et al., 1998). Items were drawn from the Conflict Tactics Scale (Straus et al., 1995) the Childhood Trauma Questionnaire (Bernstein et al., 1994; Straus et al., 1995), and Wyatt (Wyatt, 1985) The ACE questionnaire has good reliability (Cronbach alpha = .711) and validity with interview data from children who have been neglected (Wingenfeld et al., 2011).

Recent Life Stressors (RLS): In order to determine if participants had experienced major recent life stressors in the areas of health, bereavement, family relationships, social interactions, educational demands, work issues, moving house, legal challenges, and other areas during the last two weeks, 9 items were drawn from the Effects of Life Events Inventory (ELEI) (Sharpley et al., 2004), which has satisfactory validity and reliability (.741). Items of the ELEI were derived from Sarason et al. (Sarason et al., 1978) from Paykel et al.'s Distress Scale (Paykel et al., 1969) and Holmes and Rahe's Social Readjustment Rating Scale (Holmes and Rahe, 1967) and were amended to suit to the Australian setting (Tennant and Andrews, 1976).

Psychological Resilience: The Connor-Davidson Resilience Scale (CD-RISC) (Connor and Davidson, 2003) includes 25 items such as "I like a challenge", "When things look hopeless I don't give up", "I bounce back after illness or hardship", and "I am able to adapt to change". The CDRISC has been found to have five factors that measure "Personal competence, high standards and tenacity", "Trust in one's instincts, tolerance of negative affect, strengthening effects of stress", "Positive acceptance of change and secure relationships with others", "Control", and "Spiritual influences" (Connor & Davidson, 2003). Total scores on the CD-RISC are significantly correlated (.83) with total scores on the Kobasa Hardiness Measure (Kobasa, 1979) and negatively correlated (-.76) with total scores on the Perceived Stress Scale (Cohen et al., 1983), indicating high concurrent validity. The CD-RISC has acceptable reliability, ranging from 0.89 (Cronbach's alpha) to 0.87 (test-retest reliability) (Connor and Davidson, 2003).

Genotyping: Genomic DNA was isolated from buccal cells collected from participants vigorously rinsing their mouths with 15ml of commercial alcohol-free mouthwash for 1 minute. The resulting mouthwash samples were stored at room temperature. The genomic DNA was isolated using a modified method previously described by Heam and Arblaster (2010), which included centrifuging of the mouthwash sample for 1 minute at 10,000 rpm, discarding of the supernatant, adding 1.0mL of Lysis buffer to the pellet and vortexing for 20 seconds. Proteinase K (10µl of 10 mg/ml) was then added and incubated at 60°C for 10 minutes. The samples were centrifuged briefly for 10–30 seconds and the supernatant was transferred to sterile 2ml sterile tubes. Genomic DNA was precipitated by adding 100µl of 2.5M NaCl to the supernatant followed by one volume of 100% ethanol. After gentle mixing it was centrifuged at 10,000 rpm for 10 minutes. The pellet was then washed with 70% ethanol. The DNA was resuspended in 50 µl of nuclease-free water and the DNA integrity checked on 1% agarose gel. The resultant DNA samples were genotyped for HTTLPR short (s) and long (l) polymorphisms using the PCR procedure and primers described by Wendland et al. (2006) so that *ss*, *ll* and *sl* forms were identified. The PCR products were loaded onto a 1.5% agarose gel, run for 90 min at 90V in TAE buffer and visualized by ethidium bromide. All genotyping was performed in duplicate.

Statistical analysis: Data were analysed via IBM SPSS version 20. Descriptive analysis was undertaken by Frequencies and Explore to obtain means, standard deviations and 5% means to test for the effects of outliers, skewness and kurtosis. Distribution of variables was analysed by the Kolmogorov-Smirnov test plus inspection of Normal Q-Q plots. Cronbach alpha was obtained to test the internal consistency of scales used. MANOVA was used to test for gender differences across all dependent variables, and ANOVA to test for genotype and psychological variable differences on ZSDS scores. Separate linear regression equations for each 5-HTTLPR form were used to examine the relative contribution that CDRISC, Negative Childhood Events and Recent Life Stressors made to ZSDS score; Logistic regression equations tested those variables against ZSDS clinical status; Hierarchical regression and change in R square was used to test for the effect of adding variables into the regression equation. Alpha was set at .05 and observed power was determined to test for the presence of Type II errors.

All procedures were approved by the University of New England Human Research Ethics Committee.

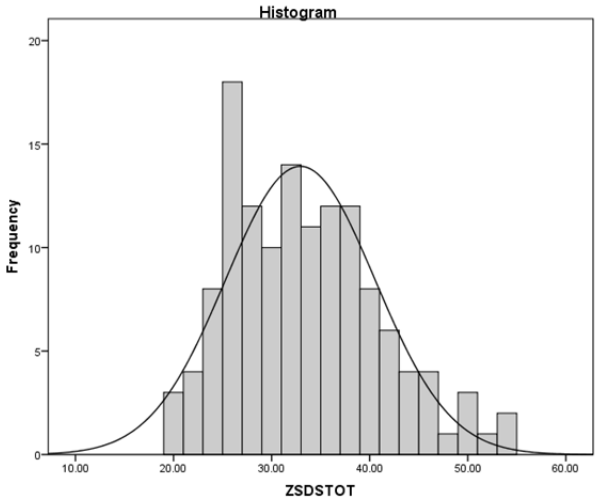
Table 1. Descriptive data (*n* = 120)

Variable	Mean	SD	Median	5% trimmed mean	Maximum	Minimum	Cronbach alpha
Age	32.53	13.49	28.0		69	17	
CDRISC	73.29	14.35	73.0	73.67	100	34	.928
ACE	7.66	8.99	5.0	6.49	43	0	.673
RLS	3.08	4.22	0.00	2.62	17	0	.653
ZSDS	32.92	7.62	32.0	32.64	53	20	.812

Results

Data. MANOVA indicated that there were no significant gender differences for genotype or any of the dependent variables shown in Table 1, thus allowing all participants' data to be examined in a single data set. Of the 65 females and 55 males in the sample, 27 were *ss*, 52 *sl* and 41 *ll* (Hardy-Weinberg Equilibrium: X2 = 2.709, p > .05). Table 1 shows the mean, SD, median, 5% trimmed mean, maximum and minimum scores for each of the variables except gender and genotype, plus the Cronbach alpha internal consistency values for the scales measuring psychological variables. Although the ACE and RLS possessed only marginal internal consistency, those values were acceptable for research purposes. The 5% trimmed means were all similar to the actual means, indicating minimal effects from outliers. Kolmogorov-Smirnov statistics were non-significant for the ZSDS and CDRISC, but there was some minor skewness in ACE and RLS. However, the Normal Q-Q plots for these scales were fairly straight lines, suggesting acceptable normality for all variables and justifying the use of parametric analyses. Although the sample's distribution of ZSDS scores did not indicate the presence of a major proportion of participants with major depression (as in some previous studies), six participants (4.8% of the sample) were currently taking antidepressants and 21 participants met Zung's criteria for clinically significant depression, including 2 of those participants who reported taking antidepressant medication. There were no significant differences in ZSDS, CDRISC, ACE or RLS scores between participants taking medication and those who were not, allowing all 120 participants to be included in further analyses. The shape of the distribution of ZSDS scores shown in Figure 1 closely approximates the normal distribution and reflects the community sample nature of the data, thus supporting the aims of the study to investigate the effects of genetic and psychological variables upon depression in the wider population.

Figure 1. Distribution of ZSDS scores across sample, plus normal distribution curve



Genotype and psychological variable effects upon depression. When examined separately, there was no significant difference in ZSDS scores according to genotype but each of the three psychological variables showed significant correlations with ZSDS scores (ACE: r = .296, p < .001; RLS: r = .349, p < .001; CDRISC: r = - .605, p < .001). However, because the aim of this study was to investigate the ways that distal and proximal stressors, plus psychological resilience, interacted with genotype to influence depression, these three psychological variables were combined with genotype as independent variables to test for the presence of significant effects on depression. A 3 (genotype) x 2 (high vs low resilience, divided according to the sample mean) x 2 (high vs low negative childhood events, mean split) x 2 (high vs low recent life stressors, mean split) ANOVA with ZSDS total score as the dependent variable indicated that there were significant effects for resilience (high CDRISC score = ZSDS score of 29.111 (5.809); low CDRISC score = 36.582 (7.536): F (1,119) = 19.603, p < .000), and a non-significant trend (at the adjusted alpha level of .05/4 = .0125) for genotype (F(2,119) = 3.971, p = .022), and there was also a trend for the interaction of ACE, RLS, genotype on ZSDS score (F = 3.329, p = .04). Observed power was sufficient to exclude Type II errors. Exploratory Scheffé post hoc comparisons across the three genotypes indicated that *sl* carriers had higher mean ZSDS scores (34.690, SD = 7.650) than *ll* carriers (31.291 (7.904): p = .037) and *ss* carriers (31.221 (6.854): p = .068), but that there was no real difference in ZSDS scores between *ss* and *ll* carriers (p = .999).

Although not significant at traditional levels, the results of these exploratory orthogonal investigations suggested that it might be valuable to further compare the ways in which the three genotypes individually interacted with ACE, RLS and CDRISC scores to influence ZDSD data, and this was undertaken via a series of regression analyses for the three genotypes to determine their individual regression equations for the relationships between ACE, RLS, CDRISC and ZSDS scores. These equations were determined for total ZSDS scores and also for ZSDS clinical status as defined by Zung and described above.

Table 2. Relationships (Beta weights) of predictor variables with ZSDS total score for each genotype

Genotype	Variable	R^2	R^2 change	Part correlation	% of R^2 accounted for	B
sl	Resilience	.393	.393**	-.606	36.72	-.328
	Negative childhood events	.492	.099**	.266	7.07	.200
	Recent stressors	.614	.122**	.349	12.1	.587
	Constant					54.196
ll	Resilience	.478	.478**	-.578	33.41	-.293
	Negative childhood events	.535	.058*	.223	4.97	.201
	Recent stressors	.545	.009	.097	0.9	.190
	Constant					51.278
ss	Resilience	.110	.110	-.331	10.95	-.201
	Negative childhood events	.112	.002	.043	1.8	.042
	Recent stressors	.112	.000	.004	.000	.009
	Constant					46.049

ZSDS total scores. Linear regression showed that the combination of ACE, RLS and CDRISC was a significant predictor of ZSDS scores for the *sl* (R square = .616) and *ll* (R square = .592) carriers, but not for the *ss* carriers (R square = .110). In addition, and as shown in Table 2, the part correlations from hierarchical regressions on each of the 3 genotype subgroups (with resilience as the first block, negative childhood experiences as the second block and recent stressors as the third block) showed the relative difference in the contribution which recent life stressors made to the R square for each regression equation.

That is, for carriers of the *sl* genotype, ZSDS total score was predicted by resilience (as a buffer against depression, as shown by the negative B values), plus recent life stressors and negative childhood events (as direct predictors of depression). By contrast, depression in *ll* carriers was best predicted by resilience (as a buffer), plus negative childhood events but not recent stressors. It should be noted that the relative power (as indicated by the size of the R square change and part correlations) of resilience was much greater than that of either distal aversive events (i.e., ACE) or proximal aversive events (i.e., RLS) for the *sl* and *ll* carriers. By using the unstandardised B coefficients shown in the final column of Table 2, approximate regression equations for depression in the *sl* and *ll* genotypes were formulated, bearing in mind the caveat that the overall equation accounts for less than 100% of the variance in ZSDS scores. Those approximate equations are:

sl: ZSDS = 54.196 + (ACE x .200) + (RLS x .587) – (CDRISC x .328).

ll: ZSDS = 51.278 + (ACE x .201) + (RLS x .190) – (CDRISC x .293).

Scatterplots and linear regression lines for these equations and for *ss* (although not significant) are shown in Figure 2.

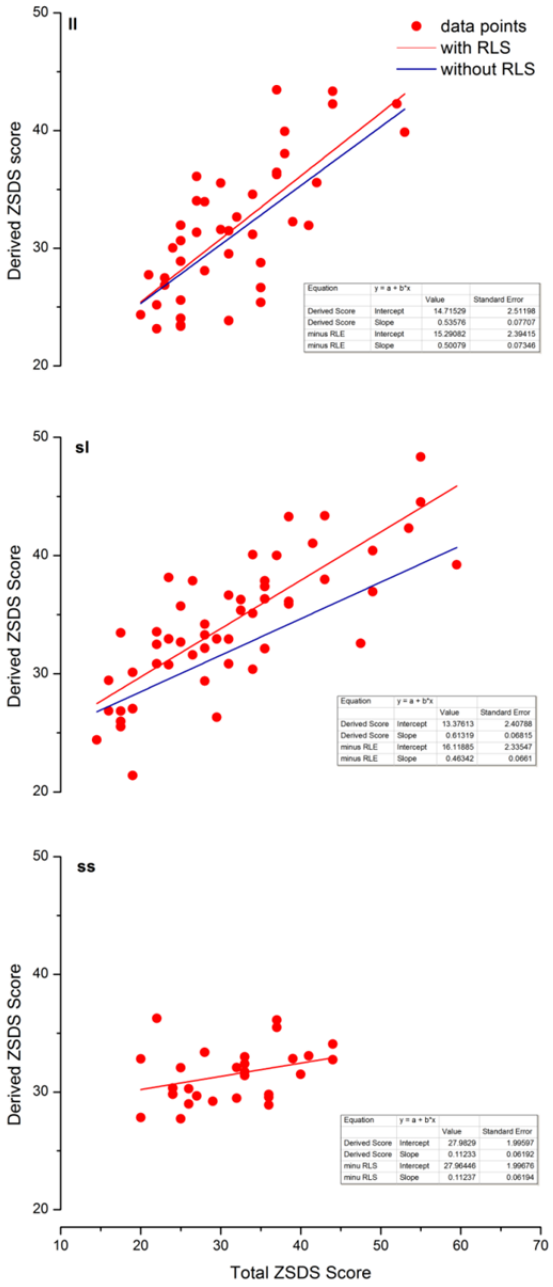
Although the RLS scores for the *sl* and *ll* genotypes were not significantly different ($F(1,96) = 2.384$, $p = .126$), the *sl* carriers' RLS scores (M = 4.132) were nearly double that for the *ll* carriers (M = 2.727) (Figure 3). When the RLS component is removed from the equations (Figure 2 blue line) the slope is significantly changed only in the *sl* group (24.4%) and not in the *ll* (6.5%) or (as expected) in the *ss* group (0%).

Negative childhood events contributed almost exactly the same amount to ZSDS scores in both the *sl* and *ll* equations, and resilience was also similar across the two equations, varying by less than 12%. The major difference between the predictive equations is for recent life stressors, where its

Table 3: Logistic regression predicting likelihood of clinically significant depression for each genotype

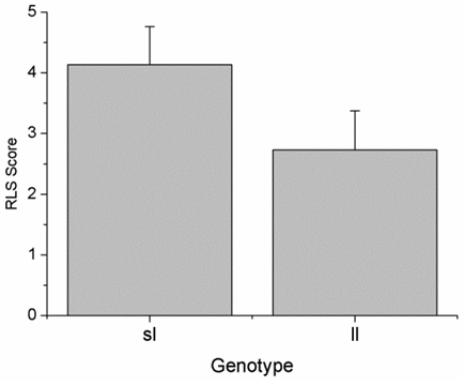
ss	*B*	S.E.	Wald	*df*	*p*	Odds Ratio	95% C.I. for Odds Ratio
ss							
CDRISC	-.045	.0492	.871	1	ns		
Childhood negative events	-.073	.122	.358	1	ns		
Recent Stressors	-6.106	.002	.000	1	ns		
Constant	2.607	3.429	.578	1	ns		
sl							
CDRISC	-.132	.050	6.923	1	.009	.867	.794–.967
Childhood negative events	.100	.051	3.905	1	.048	1.105	1.001–1.220
Recent Stressors	.247	.106	5.446	1	.020	1.281	1.040–1.576
Constant	5.205	2.913	3.193	1	ns		
ll							
CDRISC	-.111	.055	4.013	1	.045	.895	.803–.998
Childhood negative events	.115	.079	2.104	1	ns		
Recent Stressors	.119	.134	2.218	1	ns		
Constant	.210	3.790	.003	1	ns		

Figure 2: Scatter plot of the calculated ZSDS score derived from the regression analysis against the actual total ZSDS score separated into genotype. The regression line through these points is shown (red line) together with the regression line when RLS is removed from the equation (blue line)



contribution in the *sl* carriers' equation was 300% greater than that in the *ll* carriers' equation. That is, recent stressors appear to be relatively unimportant in determining depression scores for carriers of the *ll* form of the 5-HTTLPR, but hold major importance in determining depression scores for carriers of the *sl* form.

Figure 3. Comparison of sl and ll carriers' RLS scores



ZSDS clinical status

When these analyses were performed on the 'clinicity status' of participants (i.e., whether they met Zung's cutoff for clinically significant depression), results (see Table 3) were similar in that carriers of the *ss* allele showed no significant relationships between the three major predictor variables and ZSDS scores. Depression clinicity was predicted by only resilience (inversely) for carriers of the *ll* form, but all three predictor variables (resilience inversely, childhood and recent stressors directly) made significant contributions to ZSDS clinical status for the *sl* carriers.

Discussion

As in 15 of the 54 studies reviewed by Karg and colleagues with MDD or MDE samples, the *ss* form of the 5-HTTLPR was not associated with higher levels of depression than the *ll* form in this community sample of 120 males and females. Although not significant at traditional levels, carriers of the combined *sl* form had a trend towards higher depression scores compared to the *ss* or *ll* forms. These initial data on the *sl* form challenge the simple dichotomizing of the 5-HTTLPR into only *ss* and *ll* forms. Further exploration of this combined form of the serotonin transporter may therefore be justified.

The primary finding from this study was in terms of the relatively different relationships between distal and proximal stressors, resilience and depression across the three forms of the 5-HTTLPR as shown in the separate regression equations. Allowing that the *ss* form did not show any significant interaction between these variables, and accepting the key buffering role of psychological resilience against depression, the *sl* and *ll* forms appear to be differentiated by the relative power of recent stressors as predictors of depression. That is, those participants who carried a part s form (i.e., the *sl* carriers) were more influenced by recent stressors than those carriers of solely the l form. It itself, this is an argument

supporting the role of (i) both the s and l forms of the 5-HTTLPR in combination to explain the depressing effects of recent stressors, (ii) the relatively immune status of the purely l form to such recent stressors, and (iii) the apparent lack of interaction between the *ss* form and depression. This role of the combined *sl* form may be the underlying factor in the preponderance of studies reviewed by Karg and colleagues showing a significant association between stress and depression for the s form. However, because the 'pure' *ss* form was able to be contrasted with the 'combined' *sl* form in this study (but not in Karg et al.'s review), this explication of these forms and their relationship with stress and depression was a new finding. In effect, the depressive status of both *ll* and *sl* carriers was shown to be approximately equally influenced by childhood adverse events and stressors, but only the carriers of the combined *sl* form were also influenced by recent stressful events. Pure *ss* carriers appear to have not been significantly influenced by any of these stressors, and had lower depression scores than either of the other two forms.

Limitations of this study include the cultural and geographic nature of the sample, its size (although statistical power was adequate to test for orthogonal differences, a larger sample might add to the ability to detect small effects), and the nature of stressful events recorded. That is, the precise nature of recent stressors, plus the reasons why they had depressing effects upon part of the sample, require further clarification. Because of the intention to recruit a community sample rather than participants with major depression, the use of the ZSDS is satisfactory, but extension of this study via inclusion of such a clinical subsample, plus screening for the presence of other mental disorders, would enable greater generalisability of the findings reported herein.

It has been shown that, functionally, the expression of the *ll* 5-HTTLPR allele of the transporter mRNA is about three time that of the *ss* allele (Heils et al., 1996) and that the *sl* allele is somewhere between the two (Molteni et al., 2009), although there have been reports that serotonin binding or uptake is not different between the genotypes in peripheral tissues (Greenberg et al., 1999; Little et al., 1998). Simplistically, it could be expected that a decrease in the transporter protein would lead to higher concentrations of serotonin and consequently higher serotonin signaling, which is the outcome of the selective-serotonin reuptake inhibitors (SSRIs), at least in the early stage of treatment (Stahl, 1998). SSRIs are arguably the most prescribed medication for the treatment of depression and other mood disorders and, although their exact mechanism remains to be fully elucidated, it is likely that in the long term they result in a functional increase in serotonin concentrations (Bel and Artigas, 1993; Yoshioka et al., 1995), a change which is thought to be at least in part due to desensitizing the auto inhibitory receptors 5-HT1A and 5-HT1D which inhibit the 5-HT neuron firing rate (Stahl, 1998; Wong et al., 1995), thus explaining the delay in their therapeutic action.

Hence, it is understandable that there was little to no association between the *ss* allele and depression, but there was a significant association with the *ll* allele and depression in this community study. However, the finding that there was a significant association with depression between the *sl* as well as the *ll* genotype was unexpected. Further, that that association was much more strongly influenced by resilience is contradictory to some current explanations of the biological effects of these genotypes. Nevertheless, it is clear that the expression of 5-HTTLPR is also modulated by many other factors such as the glucocorticoids (Glatz et al., 2003) and BDNF (Mössner et al., 2000) (for example), all of which may influence serotonin concentrations and hence perhaps play a role in the development of depression. These findings therefore suggest that the 5-HHTLPR genotype is only part of a complex interplay between many factors, some of which remain to be identified, such as those involved in resilience traits, which in our study seemed to be the most important factor in determining the depressive influence of stressful events.

Conclusion

Overall, these results confirmed the powerful buffering role that psychological resilience plays in impeding the development of depression following distal or proximal stressors in participants that were not differentiated on the basis of 5-HTTLPR genotype. These data contribute to a growing mass of evidence that psychological resilience assists people to respond positively to stressors and resist depression, and further justifies its use in preventative training and clinical treatment settings. Further, it appears that the process of dichotomization the 5-HTTLPR into *ss* and *ll* form needs to be expanded to include the combination *sl* form, and that that form may be associated with depression and the interaction of distal and proximal stressors in more complex ways than are the *ll* or *ss* forms.

Acknowledgements

The authors acknowledge the contribution made by the Collaborative Research Network on Mental Health and Well-being in Rural Communities, supported by the Department of Industry, Innovation, Science, Research and Tertiary Education, Commonwealth Government of Australia.

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The German Journal of Psychiatry ISSN 1433-1055 http:/www. gjpsy.uni-goettingen.de
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