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VARIATION IN THE OXYTOCIN RECEPTOR GENE IS ASSOCIATED WITH INCREASED RISK FOR ANXIETY, STRESS AND DEPRESSION IN INDIVIDUALS WITH A HISTORY OF EXPOSURE TO EARLY LIFE STRESS

Amanda J. Myers, Ph.D.^{1,#}, Leanne Williams, Ph.D.^{2,3}, Justine M. Gatt, Ph.D.^{2,4}, Erica Z. McAuley-Clark, Ph.D.⁵, Carol Dobson-Stone, D.Phil.^{5,6}, Peter R. Schofield, Ph.D., D.Sc.^{5,6}, and Charles B. Nemeroff, M.D., Ph.D.¹

¹University of Miami, Miller School of Medicine, Department of Psychiatry & Behavioral Sciences, Miami, FL, USA

²Brain Dynamics Center, University of Sydney Medical School, Westmead and Westmead Millennium Institute, Sydney, New South Wales, Australia

³Department of Psychiatry and Behavioral Neuroscience, Stanford University, CA, USA

⁴Discipline of Psychiatry, University of Sydney Medical School, Sydney, New South Wales, Australia

⁵Neuroscience Research Australia, Sydney, New South Wales, Australia

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[#]Corresponding author: University of Miami, Miller School of Medicine, Department of Psychiatry & Behavioral Sciences, Rosenstiel Medical Science Building, room 6152A (D-106), 1600 NW 10th Avenue, Miami, FL 33136, ph: 305-243-3522, fax: 305-243-7522, amyers@med.miami.edu.

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Contributors:

AJM was responsible for the design, analysis and write-up of all experiments, LW manages the cohort and was involved in data collection and edited the manuscript, JMG helped with data collection and edited the manuscript, EZMC helped with data collection and edited the manuscript, CDS helped with data collection and edited the manuscript, PRS coordinated the genetic data collection and edited the manuscript, CBN edited the manuscript.

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Drs. Myers, McAuley-Clark & Dobson-Stone report no competing interests. Dr. Williams consults with Brain Resource Ltd. Dr. Gatt has previously received consultancy fees with Brain Resource Ltd for unrelated projects. Dr. Schofield reports receiving speaking fees from Janssen-Cilag. This activity does not present a conflict with the current data. Dr. Nemeroff consults for Xhale, Takeda, SK Pharma, Shire, Roche, Lilly, Allergan, Mitsubishi Tanabe Pharma Development America, Taisho Pharmaceutical Inc., Lundbeck and Prismic Pharmaceuticals. He is a stockholder in CeNeRx BioPharma, PharmaNeuroBoost, Revaax Pharma, Xhale, Celgene, Seattle genetics and Abbvie. He serves on the scientific advisory boards of the American Foundation for Suicide Prevention (AFSP), CeNeRx BioPharma (2012), the National Alliance for Research on Schizophrenia and Depression (NARSAD), Xhale, PharmaNeuroBoost (2012), the Anxiety Disorders Association of America (ADAA), and Skyland Trail. He serves on the board of directors for AFSP, NovaDel (2011), Skyland Trail, Gratitude America, and ADAA. He receives income in excess of 10,000 from PharmaNeuroBoost, CeNeRx BioPharma, NovaDel Pharma, Reevax Pharma, American Psychiatric Publishing, and Xhale. He holds two patents: Method and devices for transdermal delivery of lithium (US 6,375,990B1) and Method of assessing antidepressant drug therapy via transport inhibition of monoamine neurotransmitters by ex vivo assay (US 7,148,027B2). None of these activities present a conflict with the current data.

⁶School of Medical Sciences, University of New South Wales, Sydney, New South Wales, Australia

Abstract

Background—Oxytocin is a neuropeptide that is involved in the regulation of mood, anxiety and social biology. Genetic variation in the oxytocin receptor gene (*OXTR*) has been implicated in anxiety, depression and related stress phenotypes. It is not yet known whether *OXTR* interacts with other risk factors such as early life trauma to heighten the severity of experienced anxiety and depression.

Methods—In this study, we examined genotypes in 653 individuals and tested whether SNP variation in *OXTR* correlates with severity of features of self-reported experience on the Depression Anxiety and Stress Scale (DASS), and whether this correlation is enhanced when early life trauma is taken into account. We also assessed the effects of *OXTR* SNPs on RNA expression levels in two separate brain tissue cohorts totaling 365 samples.

Results—A significant effect of *OXTR* genotype on DASS anxiety, stress and depression scores was found and ELS events, in combination with several different *OXTR* SNPs, were significantly associated with differences in DASS scores with one SNP (rs139832701) showing significant association or a trend towards association for all three measures. Several *OXTR* SNPs were correlated with alterations in *OXTR* RNA expression and rs3831817 replicated across both sets of tissues.

Conclusions—These results support the hypothesis that the oxytocin system plays a role in the pathophysiology of mood and anxiety disorders.

Keywords

Depression; anxiety; stress; oxytocin; DASS scale; SNP; RNA expression

Background

Oxytocin (OXT) is a mammalian hormone that is best known for its role in lactation, parturition and maternal behavior. It is synthesized in the hypothalamic paraventricular and supraoptic nuclei, transported to the posterior pituitary and released into the general circulation. It is also found in extra-hypothalamic brain areas. OXT has been shown to exert effects on memory (de Wied, Diamant, & Fodor, 1993; Lerer et al., 2008a), anxiety (Heinrichs, Baumgartner, Kirschbaum, & Ehlert, 2003) and social interaction (Kosfeld, Heinrichs, Zak, Fischbacher, & Fehr, 2005; Uvnas-Moberg, 1998). OXT concentrations in plasma and cerebrospinal fluid (CSF) have been reported to have an inverse relationship with measures of aggression (Lee, Ferris, Van de Kar, & Coccaro, 2009), suicidality (Lee et al., 2009), and depression (Scantamburlo et al., 2007).

Of direct relevance to the current study, decreased CSF OXT concentrations were observed in women with a history of childhood abuse, particularly associated with emotional abuse (Heim et al., 2009). In contrast, higher plasma OXT levels are associated with higher social

anxiety symptom severity in a cohort of patients with Generalized Social Anxiety Disorder (Hoge, Pollack, Kaufman, Zak, & Simon, 2008).

Oxytocin receptors (OXTR) are localized in hypothalamic and extra-hypothalamic brain regions including the cerebral cortex. The human *OXTR* gene is located at 3p25.3 and spans approximately 17 kilobases (Kb) containing 4 exons and 3 introns (Kimura, Tanizawa, Mori, Brownstein, & Okayama, 1992). Previous work had demonstrated associations between the OXT receptor gene (*OXTR*) haplotypes and affectivity or depressive temperament (TEMPS-A scale) (Kawamura et al., 2010). In this study, we attempted to confirm and extend these findings, as well as to assess whether *OXTR* variants are associated with increased stress, depression and anxiety phenotypes particularly in the context of early life stress, using the Depression Anxiety Stress Scale (DASS).

Materials and Methods

Sample

A total of 1,226 participants in the Brain Resource International Database administered by BRAINet for scientific access (BRID; www.brainnet.net, (Gordon, 2003; Gordon, Cooper, Rennie, Hermens, & Williams, 2005)) have been assessed using the DASS measure (KEMP et al., 2005; Lovibond SH, 1995), a 42-item self report designed to measure the negative emotional states of depression, anxiety and stress. The Depression scale assesses dysphoria, hopelessness, devaluation of life, self-deprecation, lack of interest/involvement, anhedonia, and inertia. The Anxiety scale assesses autonomic arousal, skeletal muscle effects, situational anxiety, and subjective experience of anxious affect. The Stress scale is sensitive to levels of chronic non-specific arousal. It assesses difficulty relaxing, nervous arousal, and being easily upset/agitated, irritable/over-reactive and impatient. Exclusion criteria included self-report of a personal history of physical brain injury, neurological disorder, psychiatric illness or other serious medical condition including history of sleep disorder (KEMP et al., 2005). DASS scores were treated as a continuous measure in all analyses. Individuals were assessed for the number of traumatic events they had been exposed to in early life using the Early Life Stress Questionnaire (ELSQ; (Chu, Williams, Harris, Bryant, & Gatt, 2013)). The ELSQ is based on the Child Abuse and Trauma Scale, which has been shown to have strong internal consistency, test-retest reliability, and validity, as it correlates with adult outcome and psychopathology (Sanders & Becker-Lausen, 1995). The measure consists of 19 events shown to have psychological impact in childhood in previous studies (Sanders et al., 1995). The number of events was counted and analysis was performed using both the number of events as a continuous measure as well as grouping events into no traumas versus one or more traumas reported. The following criteria were met during collection of this cohort: 1) the investigation was carried out in accordance with the latest version of the Declaration of Helsinki, 2) the study design was reviewed by an appropriate ethical committee, and 3) informed consent of the participants was obtained after the nature of the procedures was fully explained.

Genotyping

Of the 1226 BRID participants with DASS measures, 682 individuals had collected DNA available for genotyping. Fifteen SNPs spanning the length of *OXTR* were selected for genotyping and ten of these SNPs passed laboratory quality control (Figure 1). Genotypes were determined using iPLEX Gold™ primer extension followed by mass spectrometry analysis on the Sequenom MassARRAY system (Sequenom, San Diego, CA) by the Australian Genome Research Facility (<http://www.agrf.org.au/>). Complete genotyping data was obtained for 653 individuals. The cohort of 653 individuals used for our analysis was 47% female, with an average age of 37 (range 6–87 years) and an average of 11 years of education (range 1–18 years). All cohort members were of Caucasian decent.

Quality Control

Prior to evaluation of samples and SNP variants, several quality control metrics were assessed. Quality metrics for samples included: 1. Removing samples with no phenotype and 2. Removing samples with call rates across all SNPs < 50%. Quality metrics for SNPs included: 1. Excluding SNPs with genotyping rates < 90% across all samples, 2. Excluding SNPs that were out of Hardy-Weinberg equilibrium (HWE) and 3. Excluding SNPs with a minor allele frequency less than 5%. A total of 7 SNPs met our criteria and were used for analysis. Three SNPs were eliminated because they were out of HWE. A total of 40% of the genetic variance of *OXTR* is captured using these 7 SNPs as measured using Haploview (Barrett, Fry, Maller, & Daly, 2005).

Imputation

To capture additional genetic variance within *OXTR*, SNPs were imputed using MaCH-Admix v2.0.198 (Liu, Li, Wang, & Li, 2013) as in Corneveaux et al (Corneveaux et al., 2010). The reference genome was the ALL GIANT.phase 1 release v3.20101123 (<http://www.sph.umich.edu/csg/abecasis/MACH/download/1000G.2012-03-14.html>) build that excludes monomorphic and singleton sites. The EUR alignment was used, which includes data from the CEPH cohorts as well as Italian, Finnish, British and Spanish samples (<http://www.1000genomes.org/category/frequently-asked-questions/population>) as is of a similar ethnic background to our cohorts. MaCH-Admix was run using the integrated (default) mode. Genotypes from 653 individuals were used for imputation, which comprised the full set of individuals for which there was both genotyping and full clinical data, including ELS, DASS scores, education, age and gender. Genotypes for 484 additional SNPs were imputed with an average quality score of 0.9 and a minimum quality score of 0.5. Imputed calls were imported into Haploview and Tagger was run to determine the minimal set of alleles which would tag the entire locus (Barrett, 2009; Barrett et al., 2005). The original genotyped SNPs were force-included in the models and pairwise tagging was performed. Fifty-one additional SNPs from this analysis were analyzed along with the original 7 polymorphisms. The average quality score was 0.8 for this set and the minimum quality score was 0.6. The minimum minor allele frequency was 2%. This set captured 90 of 90 alleles in the imputed set of SNPs at an $r^2 = 0.8$ with a max $r^2 = 0.971$.

Brain Tissue

Brain tissue was obtained from National Institute on Aging Alzheimer's disease centers, the Miami Brain Bank, the Newcastle Brain Tissue Resource, the MRC London Brain Bank for Neurodegenerative Diseases, the South West Dementia Brain Bank, The Netherlands Brain Bank and the Institut de Neuropatologia, Servei Anatomia Patologica, Universitat de Barcelona. All specimens were from donors who were greater than age 65 years at age of death. Only donors clinically classified as cognitively normal at time of death were included in this analysis. Board-certified neuropathologists performed Braak and Braak (Braak & Braak, 1995) staging on the based on the degree of neurofibrillary tangles and/or CERAD classification on the extent of neuritic plaques. Any samples with a history of other known neurological disease and or clinical history of stroke, cerebrovascular disease, Lewy bodies were excluded. Sample processing is given in references 17 and 19. The first set includes 177 samples and was 45% female, with an average age of 81 (range 65–100 years). The second set includes 188 samples and was 48% female, with an average age of 80 (range 65–102 years). All cohort members were of Caucasian decent.

Expression Profiling

Two data series were used to examine *OXTR* SNP genotypes and changes in *OXTR* transcript expression. Set 1 included data freely available at (<http://labs.med.miami.edu/myers/LFuN/LFuN.html>, (Webster et al., 2009)). This set includes 177 samples of human brain tissue from temporal, frontal or parietal cortex from individuals free of major neuropathology. Transcriptome profiles from this series were obtained on an Illumina Ref-seq8 microarray. The second series is a set of 188 pathology-free samples collected using the same criteria as set 1 (Zhang et al., 2013). Transcriptome profiles from this series were obtained on an Illumina HT-12 microarray. All transcript profiles were corrected as in Webster et al (Webster et al., 2009) with the exception that the lumi R package was used for set 2 (Du, Kibbe, & Lin, 2008). SNP genotypes from both sets were imputed using the same procedure as before, and Tagger was used as before to define minimal sets of variants to test. For both sets, genotypes for 484 additional SNPs were imputed with an average quality score of 0.95 and a minimum quality score of 0.54. Tags were determined independently for each set using Tagger. For the first expression set, 41 variants were used as tags with an average quality score of 0.8 and a minimum quality score of 0.6. The minimum minor allele frequency was 2%. This set captured 124 of 124 alleles in the imputed set of SNPs at a r^2 0.8 with a max r^2 =0.941. For the second expression set, 70 variants were used as tags with an average quality score of 0.9 and a minimum quality score of 0.6. The minimum minor allele frequency was 2%. This set captured 166 of 166 alleles in the imputed set of SNPs at an r^2 0.8 with a max r^2 =0.972.

Analysis

Several tests were performed in the BRAINnet data series. TEST 1: We use Student's t-tests to examine whether there were differences in the DASS anxiety, DASS stress or DASS depression scores between individuals that experience one or more ELS events versus those that had no ELS. TEST 2: We examined whether there were any covariates that contributed to the relationships between ELS and DASS scores. TEST 3: We tested models including

the main effects of *OXTR* SNPs, significant covariates from TEST 2, an interaction term for the joint effects of ELS and SNP genotype. We modeled DASS score as our outcome and regressed both SNP genotype and the interaction between SNP genotype and ELS. TEST 4: We performed an analysis in a separate dataset from neuropathologically normal human brains to examine whether any of the significant SNPs in our tests had a downstream functional effect, specifically testing whether they correlated with *OXTR* transcript expression changes. For all models the outcomes were DASS scores (either anxiety, stress or depression). Analysis for TEST 1 and TEST 2 was performed using SPSS v21 (Predictive Analytics SoftWare) using Student's t-tests, and general linear models (GLM). Tests were corrected for multiple testing using a bootstrap procedure with 1000 permutations. Analysis for TEST 3 was performed using Plink v1.06 (Purcell et al., 2007) using an adaptive permutation procedure to correct for testing across multiple SNPs. SNP p-values were additionally corrected for multiple testing across the 3 different clinical phenotypes using a Bonferroni method. Expression data was analyzed for TEST 4 using Plink v1.06 (Purcell et al., 2007) using a linear model with permutation to correct for multiple testing.

Results

Symptoms and Early Life Stress

For TEST 1, we found a greater number of traumatic events was associated with greater severity of symptoms for stress (p-value=7.604E-09), anxiety (p-value=3.604E-06) and depression symptom scores (p-value=2.245E-11). These results are shown in Figure 2.

Symptoms-ELS relationship and Covariates

For TEST 2, we found that none of our demographic variables (gender, age or years of education) were significant covariates (Table 1A) when corrected for multiple testing. Additionally, none of these variables significantly interacted with ELS (Table 1B). Therefore, gender, age and years of education were not included in the modeling of SNP effects.

Symptoms and SNPs

We were interested in examining two outcomes in our analysis of *OXTR* SNPs. In TEST 3, we sought to determine whether there was a main effect of SNP genotype on symptoms, assessed by DASS score and additionally, we examined whether there was an effect of ELS on the genotype and symptom relationship. Significant results for both of these tests are shown in Table 2. We found significant main effects of SNP genotype both on DASS stress and depression scores, but not with DASS anxiety. When ELS was considered there were significant SNPs identified for each type of symptom. The most interesting SNP in our findings was rs139832701 that gave significant results when ELS was considered in the models of DASS stress and depression, but just failed to survive correction for DASS anxiety. Additionally, there were main effects of this SNP on both stress and depression scores. The variant rs139832701 is a rare SNP just upstream of *OXTR* (Figure 3).

OXTR genotypes and Expression

Because rs139832701 is located upstream of *OXTR*, it was of interest to determine whether this variant changes *OXTR* transcript expression. In TEST 4, we used two different cohorts including subjects of European ancestry who were verified as pathologically normal. These samples were analyzed as previously (Webster et al., 2009) to determine whether there was a linear relationship between *OXTR* allele dose and *OXTR* transcript levels. Results are shown in Table 3. There were several SNPs in each human brain tissue set where there was a linear relationship between *OXTR* expression and SNP genotypes. The clinically associated variants (rs139832701 and rs11131147) were not significant in our expression analysis, indicating it is likely that these variants do not act by changing *OXTR* levels. However, one common SNP was significant in both brain tissue sets. rs3831817 showed significantly different expression in each allele group as shown in Figure 4. The location of rs3831817 with respect to rs139832701 is shown in Figure 3.

Discussion

The physiological role of oxytocin has been intensively investigated. Initial work demonstrated its role in labor and lactation (Thorburn & Challis, 1979). More recent work has focused on oxytocin's effects on human social behavior (Ebstein, Israel, Chew, Zhong, & Knafo, 2010) including work demonstrating that intranasal administration of oxytocin increases trust in humans (Kosfeld et al., 2005) and is a potent antagonist of amygdala activation and brainstem activation in autonomic and behavioral manifestations of fear (Kirsch et al., 2005). Further work has now defined a role for oxytocin in symptoms of depression (Malik, Zai, Abu, Nowrouzi, & Beitchman, 2012) and in risk factors for depression, such as early life stress (Bakermans-Kranenburg & van Ijzendoorn, 2008; Rodrigues, Saslow, Garcia, John, & Keltner, 2009). Oxytocin has also been implicated in other maladaptive emotional traits including aggression (Malik et al., 2012) and autistic behaviors (Andari et al., 2010).

In this study we focused on genetic variation in *OXTR* and whether early life stress exposure moderates the relationship between *OXTR* and symptoms of depression and anxiety. We found a significant interaction in which individuals with the *OXTR* rs139832701 variant who were also exposed to stress showed significantly more severe symptoms of depression and related symptoms of stress and (at trend level) anxiety. These findings suggest that genotypes of this *OXTR* variant might in the context of early life stress increase risk for expression of symptoms that lead to a much higher likelihood of developing mood and anxiety disorders.

To our knowledge the present findings are the first report on an association between early life stress, mood and anxiety symptoms and the *OXTR* rs139832701 variant. It extends on previous reports in which *OXTR* has implicated a haplotype comprised of 7 variants (rs11131149, rs2243370, rs2243369, rs113316193, rs2254298, and rs2268491) with depressive temperament. Mapping these prior results onto the effects seen in our screen (Figure 5) shows the SNP associated with DASS scores (rs139832701) is in linkage equilibrium with the prior SNPs (r^2 values < 0.8), suggesting this is perhaps an independent result from prior observations. Other work has focused on a variant (rs53576) in the third

intron of the *OXTR* that has been associated with greater empathy and stress reactivity (Rodrigues et al., 2009), greater maternal sensitivity (Bakermans-Kranenburg et al., 2008), higher self esteem and optimism (Saphire-Bernstein, Way, Kim, Sherman, & Taylor, 2011), differing cortisol levels during the Trier Social Stress Test (Chen et al., 2011), differences in positive affect (Kim et al., 2010) and the development of future depressive symptoms in the context of childhood maltreatment (McQuaid, McInnis, Stead, Matheson, & Anisman, 2013). This variant is also in linkage equilibrium with our associated SNP ($r^2=0.14$), again suggesting our results are independent from prior studies.

There are some limitations to our results as reported. First, our DASS measures are all self-report. This might increase the noise in our clinical measures as outcomes are not validated by clinicians or examinations of medical records. Secondly, our expression cohort and our DASS cohort are sampled from different populations. While they are well balanced for gender distributions (DASS cohort: 47% female, Expression set 1: 45% female, Expression set 2: 48% female), the expression set cohorts are both considerably older than the DASS series. This is in part due to the fact that expression profiles might be affected by causes of death in younger samples, i.e. the CDC reports the major cause of death in younger cohorts is unintentional injury, which is likely to result in more head trauma than heart disease, which is the major cause of death in the elderly (http://www.cdc.gov/injury/wisqars/pdf/10lcid_all_deaths_by_age_group_2010-a.Pdf). Head trauma and similar injuries will affect brain expression profiles, thus it is less ideal to use younger brain tissue samples to perform expression profiling in control samples. The final limitation of this paper is that we were not able to map a downstream effect of the *OXTR* rs139832701 variant.

While we were not able to map a downstream change in *OXTR* gene expression to SNPs that were associated with differences in DASS outcomes, we were able to map one SNP (rs3831817) that was associated with differences in *OXTR* transcript expression in two different human brain tissue sets. rs3831817 is in moderate linkage disequilibrium with our DASS effect SNP (rs139832701, $r^2=0.74$, Figure 5); however, again rs139832701 was not associated with differences in expression in two separate datasets (Table 3). rs3831817 falls into a cluster of SNPs that have been associated with autism risk in Chinese Han (Wu et al., 2005), Japanese (Liu et al., 2010) and Israeli (Lerer et al., 2008b) cohorts. Mapping this SNP onto the effects seen in prior screens (Figure 5) showed strong linkage disequilibrium between prior results and our expression SNP rs3831817 (r^2 values = 0.8 for rs53576 and rs237897) indicating that perhaps the prior associated SNPs were having effects through changes in downstream transcript levels. Other studies have linked epigenetic silencing and deletions of *OXTR* to the exhibition of autism (Gregory et al., 2009) suggesting that *OXTR* expression might be crucial to autism and rs3831817 might warrant further study in this context.

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Figure 1. Genotyped SNP locations and linkage disequilibrium

1A. Linkage disequilibrium map of SNPs chosen for genotyping. Linkage disequilibrium was calculated using data using Haploview and the CEPH data from the hapmap project (<http://hapmap.ncbi.nlm.nih.gov/>). The SNPs that failed to pass QC were rs237894, rs237895, rs2268496, rs237900 and rs237902. **1B.** Basepair positions of SNPs used for analysis relative to the *OXTR* gene.

Figure 2. DASS scores comparing individuals with and without early life stress

Shown are the distributions of DASS scores for individuals with less than one early life stress trauma (ELS <1) or greater than one early life stress trauma (ELS ≥ 1) for each of the 3 DASS measures (DASS_STRESS, DASS_ANXIETY or DASS_DEPRESSION). Significant differences were obtained for all scores, indicating that ELS impacts adult stress, anxiety and depression outcomes.

Figure 3. Location of rs139832701 and rs3831817 with respect to *OXTR*

Shown in the figure is the location of rs139832701 and rs3831817 from data from the UCSC genome mapping site (<http://genome.ucsc.edu>).

Figure 4. Expression of *OXTR* by rs3831817 genotypes

Shown in the figure is a boxplot of rs3831817 genotypes (x-axis) and *OXTR* transcript levels (y-axis) for each brain profiling set. The adjusted linear p-values comparing allele dose and expression across all genotypes were 0.01113 (set 1) and 0.000721 (set 2).

Figure 5. Linkage disequilibrium plot of prior and current results

Shown are the r^2 values for SNPs previously associated with depression and anxiety and the results from our current screen.

Covariate analysis

Table 1

Table 1A lists p-values for the comparisons of gender, age and years of education with each of the DASS components. Table 1B lists the p-values for the interaction between ELS and each covariate.

	GENDER	BOOTSTRAP	AGE	BOOTSTRAP	EDUCATION	BOOTSTRAP
ANXIETY	0.181	0.958	1.96E-04	0.544	0.003	0.371
DEPRESSION	0.729	0.515	0.137	0.566	0.693	0.422
STRESS	0.761	0.333	0.435	0.549	0.172	0.399

SNP analysis

Table 2

Table 2 lists p-values for the comparisons of SNP genotype and ELS with each of the DASS components. RAW lists the unadjusted p-values from either the test of SNP genotype against DASS score (MAIN EFFECTS) or the unadjusted p-values from a linear test of whether early life stress (ELS) significantly interacted with the SNP genotype and DASS phenotype relationship (ELS INTERACTION). SNP ADJUSTED are the p-values adjusted for testing 58 variants using the bootstrap procedures in Plink. CORRECTED corrects the SNP ADJUSTED p-values for the 3 different DASS score tests using a Bonferroni correction. Only significant SNPs are shown.

ANXIETY										
MAIN EFFECTS			ELS INTERACTION							
SNP	RAW	SNP ADJUSTED	CORRECTED	FREQ	HWE	SNP	RAW	SNP ADJUSTED	CORRECTED	FREQ HWE
rs237886	0.028	0.036	0.107	0.444	0.979	rs59190448	0.022	0.019	0.058	0.190 0.018
						rs139832701	0.051	0.023	0.068	0.016 1.000
						rs17297971	0.011	0.333	N/A	0.047 0.460
						rs237924	0.036	0.438	N/A	0.062 0.142
						rs7652281	0.045	0.857	N/A	0.062 0.142

STRESS										
MAIN EFFECTS			ELS INTERACTION							
SNP	RAW	SNP ADJUSTED	CORRECTED	FREQ	HWE	SNP	RAW	SNP ADJUSTED	CORRECTED	FREQ HWE
rs11131147	0.014	0.016	0.047	0.068	0.815	rs139832701	0.007	0.005	0.016	0.015 1.000
rs7652281	0.038	0.049	0.148	0.062	0.142	rs7652281	0.016	0.857	N/A	0.062 0.142
rs237892	0.054	0.065	N/A	0.071	0.059	rs237892	0.029	0.356	N/A	0.071 0.059
						rs190216	0.035	0.214	N/A	0.079 0.025

DEPRESSION										
MAIN EFFECTS			ELS INTERACTION							
SNP	RAW	SNP ADJUSTED	CORRECTED	FREQ	HWE	SNP	RAW	SNP ADJUSTED	CORRECTED	FREQ HWE
rs139832701	0.004	0.010	0.011	0.015	1.000	rs139832701	0.001	0.004	0.011	0.015 1.000
rs11131147	0.015	0.019	0.058	0.068	0.815					

DEPRESSION											
MAIN EFFECTS						ELS INTERACTION					
SNP	RAW	ADJUSTED	CORRECTED	FREQ	HWE	SNP	RAW	ADJUSTED	CORRECTED	FREQ	HWE
rs13093809	0.035	0.049	0.147	0.047	0.437						

Table 3**Expression analysis**

Table 2 lists p-values for the comparisons of SNP genotype and *OXTR* expression. RAW lists the unadjusted p-values from comparisons of SNP genotype against expression. SNP ADJUSTED are the p-values adjusted for testing multiple variants using the bootstrap procedures in Plink. Only significant SNPs are shown with the exception of rs139832701 and rs1131147 since these two SNPs gave significant results in our analysis of DASS scores.

EXPRESSION SET 1				
MAIN EFFECTS				
SNP	RAW	SNP ADJUSTED	FREQ	HWE
rs237881	0.005677	0.006168	0.419	0.5559
rs237876	0.009118	0.01275	0.28	0.8047
rs3831817	0.009919	0.01113	0.38	0.6681
rs2268498	0.01505	0.0142	0.402	0.0235
rs62243375	0.01541	0.01242	0.06	0.5017
rs237883	0.03678	0.03411	0.056	0.6064
rs139832701	0.9383	1	0.139	0.0065
rs1131147	0.9081	0.8571	0.103	0.4705

EXPRESSION SET 2				
MAIN EFFECTS				
SNP	RAW	SNP ADJUSTED	FREQ	HWE
rs7629329	1.93E-07	0.000001	0.259	0.0953
rs11718289	8.07E-07	0.000002	0.426	0.1434
rs237902	1.88E-06	0.000009	0.349	0.6313
rs34955659	1.08E-05	0.000017	0.023	1
rs237888	8.64E-05	0.0001127	0.057	1
rs34055574	0.0001853	0.0002057	0.339	0.5512
rs62242632	0.0002328	0.0002299	0.103	0.4094
rs6443206	0.0002205	0.0002393	0.234	0.8965
rs9878427	0.0003801	0.0003105	0.113	1
rs11476	0.000303	0.0004044	0.339	0.0042
rs237894	0.0004674	0.0005243	0.288	1
rs3831817	0.000644	0.000721	0.389	0.9276
rs2268492	0.001291	0.001518	0.329	0.9922
rs237889	0.002488	0.002914	0.379	0.7455
rs2139184	0.004696	0.00535	0.031	1
rs237917	0.005762	0.006785	0.284	0.742
rs114641034	0.01002	0.007115	0.035	1
rs11711703	0.00428	0.00833	0.174	0.7371
rs2268495	0.004871	0.01014	0.225	0.5321

EXPRESSION SET 2				
MAIN EFFECTS				
SNP	RAW	SNP ADJUSTED	FREQ	HWE
rs237918	0.01105	0.01408	0.473	0.9736
rs7635237	0.02051	0.0185	0.079	1
rs190216	0.02186	0.02687	0.076	0.1948
rs34474424	0.03345	0.0348	0.085	0.952
rs56672907	0.0296	0.03622	0.207	0.0033
rs73132859	0.03383	0.04492	0.046	1
rs139832701	0.2099	0.1626	0.124	0.0026
rs11131147	0.2322	0.2535	0.146	1