Neuropeptide S receptor gene (NPSR) and life events: G × E effects on anxiety sensitivity and its subdimensions

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Abstract
Objectives. The pathogenesis of anxiety is assumed to be interactively influenced by genetic and environmental factors. Thus, a gene–environment interaction (G × E) study of the neuropeptide S receptor gene (NPSR) A/T polymorphism (rs324981) and life events was conducted with respect to anxiety sensitivity (AS) as an intermediate phenotype of anxiety disorders.
Methods. A sample of 475 healthy German subjects was genotyped for NPSR and assessed for AS, childhood maltreatment (CTQ) and recent life events (LTE). Influences on AS and its subdimensions were determined by a step-wise hierarchical regression and a multiple indicator multiple cause (MIMIC) model.
Results. Significant main effects of NPSR and CTQ as well as significant G × E were observed, with T/T homozygosity and a high CTQ score resulting in increased anxiety sensitivity. MIMIC modelling yielded association of AS subfactor “concern about mental/cognitive incapacitation” and the basal somatic subdimension “concern about physical sensations” to be associated with CTQ and its interaction with NPSR, while the acute somatic subfactor “concern about heart/lung failure” was associated with NPSR and its interaction with LTE.
Conclusions. Results indicate G × E effects of the more active NPSR rs324981 T allele and life events on AS with differential effects of temporally proximal and distal factors on specific AS subdimensions.

Key words: Gene–environment interaction, neuropeptide S, NPSR, childhood trauma, threatening life events, anxiety sensitivity

Introduction
Neuropeptides are discussed to play a crucial role in the etiology of anxiety and anxiety disorders, with particular evidence for the neuropeptide S (NPS) system (Reinscheid and Xu 2005; for review see Okamura and Reinscheid 2007; Pape et al. 2010). NPS is a 20-amino acid peptide, which acts as an agonist at the G-protein coupled NPS receptor (NPSR) to increase free intracellular Ca²⁺ and cAMP (Xu et al. 2004; Reinscheid and Xu 2005; Okamura and Reinscheid 2007). In rodent models, centrally administered NPS causes anxiolytic-like effects in tests like open field or elevated plus maze (Xu et al. 2004; Rizzi et al. 2008; Wegener et al. 2011), while in turn NPSR knock-out mice were observed to exhibit increased anxiety-like behaviour (Duangdao et al. 2009).

The human NPSR gene (7p14.3) is located in a chromosomal region (7p14–15), which has previously been found to be linked to panic disorder (Knowles et al. 1998; Crowe et al. 2001; Logue et al. 2003). A functional NPSR A/T single nucleotide polymorphism (rs324981) causing an amino acid exchange from Asn to Ile (N107T) has been reported to be of functional relevance with the T allele (Ile107) increasing NPSR expression and NPS efficacy at NPSR about tenfold (Bernier et al. 2006; Reinscheid et al. 2005). This more active T allele has been found to be associated with panic disorder in several independent samples (Okamura et al. 2007; Donner et al. 2010; Domschke et al. 2011). Furthermore, significantly elevated anxiety sensitivity and increased autonomic arousal during a behavioural avoidance test were observed in NPSRT allele carriers (Domschke...
et al. 2011), and imaging genetic studies yielded evidence for the more active NPSRT allele to be associated with decreased cortical and increased amygdalar activity during processing of anxiety-relevant emotional stimuli in patients with panic disorder and healthy controls, respectively (Dannlowski et al. 2011; Domschke et al. 2011). Association of this gain-of-function NPSR rs324981 T allele with panic disorder and related symptoms in humans seems counterintuitive when considering rodent models, where NPS and NPSR agonists have been shown to rather exert a dose-dependent anxiolytic effect (cf. Xu et al. 2004). A possible explanation of this paradox might be that panic disorder and panic-associated symptoms are to a great extent conferred via an increased level of arousal (e.g., Clark 1986; Bouton et al. 2001; Blechert et al. 2007), which in animal models has been found to be driven by increased NPS activity with a potentially differential dose-dependent effect on arousal and anxiety, respectively (Xu et al. 2004; Reinscheid et al. 2005; Leonard et al. 2008; Rizzi et al. 2008).

Besides genetic factors, environmental influences such as abuse (Brown and Harris 1993; Stein et al. 1996; e.g., Bandelow et al. 2002), loss/separation experiences in childhood (e.g., Kendler et al. 1992; Bandelow et al. 2001) or recent stressful life events (Faravelli 1985) have a well-documented impact on the pathogenesis of anxiety disorders, with some overlapping effects across the anxiety disorder spectrum as well as on comorbid major depression (cf. Klaue et al. 2010). Also, the intermediate phenotype of anxiety sensitivity (AS) has been reported to be predicted by exposure to parental dyscontrol in childhood related to negative emotional states (Watt and Stewart 2003) and emotional maltreatment in childhood (Scher and Stein 2003).

As anxiety and anxiety disorders in particular are complex genetic traits with an interaction of genetic and environmental factors, the exploration of gene–environment interaction (G × E) is germane to the dissection of the pathogenetic mechanisms of anxiety. In humans, however, so far only very few studies have been dedicated to G × E in anxiety (see Nugent et al. 2011), mostly focussing on variation in the serotonin transporter gene (Stein et al. 2008; Laucht et al. 2009; e.g., Grabe et al. 2009; Klaue et al., 2011). Recently, the rs16147 polymorphism in the neuropeptide Y (NPY) gene has been reported to impact generalized anxiety disorder after hurricane exposure (Amstadter et al. 2010) and to increase HPA axis responses to acute psychosocial stress in interaction with early life adversity (Witt et al. 2011). With respect to neuropeptide S gene variation, however, to the best of our knowledge no G × E study has been conducted yet in anxiety-related phenotypes, while a rodent study strongly suggests an interaction of stress and the neuropeptide S system in the mediation of stress-/anxiety-related phenomena (Ebner et al. 2011).

Thus, in the present study we set out to elucidate the interactive influence of NPSR rs324981 A/T gene variation and critical environmental factors such as childhood maltreatment and recent threatening life events on anxiety sensitivity (AS), an intermediate phenotype of anxiety disorders measuring the cognitive vulnerability to anxiety. AS reflects fear of anxiety symptoms (Reiss et al. 1986) and is suggested as a predictor of anxiety disorders, especially panic disorder (Schmidt et al. 1997, 1999; Schmidt et al. 2006). Additionally, AS subdimensions have been proposed to serve as particularly sensitive intermediate phenotypes for disentangling genetic, environmental and G × E effects on anxiety (cf. Klaue et al. 2011).

Methods

Samples and procedures

A sample of healthy participants (N = 475; f = 343, m = 132; mean age: 25.2 years, SD: 6.2 years) was consecutively recruited at the Departments of Psychiatry, Muenster and Wuerzburg, Germany. Initially, general criteria for participation (Caucasian background, fluency in German) were checked in a telephone-interview. Manifest mental Axis I disorder, pregnancy, severe medical conditions, and use of illegal drugs were exclusion criteria. In the subsequent test session, sociodemographic data were ascertained and drug abstinence was tested with a urine drug screen test. Mental Axis I disorders were excluded using a structured diagnostic interview (Lecrubier et al. 1997), performed by experienced clinical psychologists or psychiatrists. Participants filled in a set of psychological measures (see below). A blood sample (20 ml EDTA blood) was taken for genetic analyses. Participants received 50 remuneration. The study was approved of by the ethic committees of the Universities of Muenster and Wuerzburg, Germany. Written informed consent was obtained from all subjects.

Self-report measures

Anxiety Sensitivity Index (ASI). The German version of the ASI (Reiss et al. 1986; Alpers and Pauli 2001) comprises 16 items, combined with five-point Likert type -scales. Most factoranalytic studies suggest three- or four-factor solutions of the ASI (cf. Blais et al. 2001).

Childhood Trauma Questionnaire (CTQ). The German version of the CTQ (Bernstein and Fink 1998;
Gast et al. 2001; Wingenfeld et al. 2010) comprises 28 items with five-point Likert answer scales, designed to retrospectively assess negative childhood experiences.

List of Threatening Experiences (LTE). The LTE (Brugha et al. 1985) assesses the experience of life events in 12 categories during a time period of the past 12 months.

Genotyping

The sample was genotyped for the NPSR rs324981 A/T (Asn107Ile) polymorphism according to published protocols (Domshke et al. 2011). Genotypes were determined by investigators blinded for pheno- and independently by two investigators. Hardy–Weinberg criteria, assessed with the online available program DeFinetti (Wienker and Strom, accessed May 2011), were fulfilled for NPSR genotype distribution in the present sample (T/T: 20.8%, A/T: 51.6%, A/A: 27.6%; \( P = 0.43 \)).

Statistical analysis

For genetic analyses, two variables were created (variable a: 0.5 = T/T, −0.5 = A/T and −0.5 = A/A; variable b: −0.5 = T/T, −0.5 = A/T and 0.5 = A/A). Variable a (T/T vs. A allele carriers) reflects a recessive model, variable b (A/A vs. T allele carriers) a dominant model when assuming T to be the risk allele, or vice versa when assuming A to be the risk allele. As suggested by Kraemer and Blasey (2004), CTQ and LTE sum scores were centred (means = 0) in order to prevent statistical inference errors. To exclude possible objectionable confounding effects, gene–environment correlations (rGE) were analyzed using bivariate correlation analysis. Gender was coded 0.5 for females and −0.5 for males.

The effects of NPSR genotype, negative childhood experiences (CTQ), recent life events and their interaction (G \( \times \) E) on AS (ASI), respectively, were estimated with hierarchical multiple regression, using SPSS 18.0. For all tests, a \( P \) value < 0.05 was considered statistically significant. Analyses were run in three steps: The first included gender, NPSR genotype, CTQ sum score and LTE sum score in order to evaluate the main effects of these variables on AS. Analyses were run twice with both operationalizations of genotype (T/T vs. A/T and A/A; A/A vs. A/T and T/T). In the second step, G \( \times \) E interaction terms for NPSR genotype with CTQ and LTE, respectively, were added. Thirdly, three two-way interaction terms for gender with NPSR genotype, CTQ and LTE, respectively, were included, as well as two three-way interaction terms for gender, NPSR genotype and CTQ or LTE, respectively. Significance of increase of explained variance (\( R^2 \)) over successive regression steps was tested by means of the \( F \)-test. The assumption of linear regression that residual variances are Gaussian distributed was checked by a post-hoc Kolmogorov–Smirnov test and could be corroborated for each regression step. According to a post-hoc power calculation of multiple regression there was sufficient power (> 0.90) to explain 5% of ASI variance with a type I error rate of 0.05.

Because of the ambiguous factor structure of the ASI (see above), we determined the dimensional structure of ASI answers of our sample by computing and comparing different factor solutions proposed in the literature using confirmatory factor analyses (CFA). To elucidate the influence of gender, NPSR genotype, CTQ and LTE, we again involved these predictors in the best fitting ASI measurement model, yet not as predictors of observed ASI sum scores but of distinct latent AS subdimensions. This approach of a Multiple Indicator Multiple Cause (MIMIC) analysis has previously been applied by Klauke et al. (in press). All CFA and MIMIC analyses were conducted with Mplus 5.21 (Muthén and Muthén 2007), relying on robust mean and variance adjusted weighted least-squares (WLSMV) estimations. For statistical evaluation of overall model fit \( \chi^2 \)-values will be reported, as descriptive fit indices the Comparative Fit Index (CFI), the Tucker Lewis Index (TLI), and the Root Mean Square Error of Approximation (RMSEA) are used.

Results

Descriptive data

Descriptive statistics for the 475 participants, including NPSR genotype distribution, are given in Table I.

Hierarchical multiple regression with ASI sum scores

Figure 1 illustrates the relation of NPSR genotype, CTQ and LTE sum scores to ASI sum scores based on a linear regression analysis.

Genetic operationalization a (NPSR T/T vs. A allele carriers). A significant main effect of T/T genotype (\( \beta = 0.09, t = 2.00, P < 0.05 \)) and childhood maltreatment (CTQ) (\( \beta = 0.10, t = 2.05, P = 0.04 \)) on AS was observed in step 1, while neither recent threatening experiences (LTE) (\( P = 0.89 \)) nor gender (\( P = 0.09 \)) directly affected ASI sum scores. In step 2, a significant interaction between T/T (vs. A/T
Table I. Descriptive characteristics of study participants stratified by NPSR rs324981 A/T genotype.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total sample</th>
<th>A/A</th>
<th>A/T</th>
<th>T/T</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
<td>SD</td>
<td>N</td>
</tr>
<tr>
<td>Age (years)</td>
<td>475</td>
<td>25.21</td>
<td>6.15</td>
<td>131</td>
</tr>
<tr>
<td>ASI (sum)</td>
<td>475</td>
<td>13.68</td>
<td>6.75</td>
<td>131</td>
</tr>
<tr>
<td>CTQ (sum)</td>
<td>475</td>
<td>33.07</td>
<td>6.74</td>
<td>131</td>
</tr>
<tr>
<td>LTE (sum)</td>
<td>475</td>
<td>1.40</td>
<td>1.23</td>
<td>131</td>
</tr>
</tbody>
</table>

ASI, Anxiety Sensitivity Index (sum score); CTQ, Childhood Trauma Questionnaire (sum score); LTE, List of Threatening Experiences (sum score); all comparisons between NPSR genotypes were not significant at a significance level of \( p < 0.05 \).

Figure 1. Linear regression analysis of CTQ and LTE sum scores on ASI sum score stratified by NPSR rs324981 A/T genotype.

and A/A) and CTQ (\( \beta = 0.17, t = 2.66, P < 0.01 \)) on ASI values was obtained as well as a still significant impact of T/T genotype (\( \beta = 0.09, t = 2.05, P < 0.04 \)) and CTQ (\( \beta = 0.21, t = 3.32, P < 0.01 \)). The addition of G \( \times \) E interaction terms in step 2 accounted for a significant increment in explained variance (\( R^2 = 0.043, \Delta R^2 = 0.020, \Delta F(2,468) = 4.94, P < 0.01 \)). Although interactive influence of LTE and NPSR T/T genotype on ASI is visually suggested (see Figure 1), regression analysis did not yield a statistically significant \( G \times E \) effect between these predictors in step 2 (\( \beta = 0.05, t = 0.98, P = 0.33 \)). None of the interactions with gender involved in step 3 was significant (data not shown). Accordingly, step 3 did not increase explained variance regarding NPSR gene variation and CTQ significantly (\( \Delta R^2 = 0.010, \Delta F(5,463) = 0.99, P = 0.43 \)).

Gene–environment correlations (rGE)

No significant gene–environment correlations (rGE) between the two environmental predictors and the two genetic operationalizations, respectively, were observed (data not shown).

Factor analysis (CFA) of Anxiety Sensitivity Index/MIMIC model

A confirmatory factor analysis showed a sufficiently fitting model (\( \chi^2 = 209.36, df = 60, P < 0.00005, \)
MIMIC modelling. The four factors are shown in Figure 2.

The MIMIC model analysis contained the four identified ASI subdimensions as dependent variables and gender, NPSR, CTQ, LTE as well as G × E as predictors. Based on the significant results yielded in the hierarchical regression analysis, in this analysis only genetic operationalization a (T/T vs. A/T and
A/A) was taken into account, and no interaction effects with gender (cf. regression step 3) were included.

According to this adequate model-fit (see Figure 2; χ² = 201.86, df = 82, P < 0.00005, CFI = 0.91, TLI = 0.93, RMSEA = 0.055), the subdimension “concern about physical sensations” was positively influenced by gender (β = 0.14, P < 0.01), CTQ sum score (β = 0.21, P < 0.01) and its interaction with NPSR T/T genotype (β = 0.23, P < 0.01). The subdimension “concern about heart/lung failure” was significantly influenced by gender (β = 0.15, P < 0.01), NPSR T/T genotype (β = 0.12, P = 0.04) and its interaction with the LTE sum score (β = 0.13, P = 0.05). For the subdimension “concern about mental/cognitive incapacitation”, significant influences of CTQ sum score (β = 0.31, P < 0.01) and its interaction with NPSR T/T genotype (β = 0.18, P = 0.02) were discerned. The factor “concern about loss of control” was only influenced by gender (β = −0.13, P = 0.05) (see Table II).

### Discussion

In the present G × E study, we observed significant main effects of the functional neuropeptide S receptor gene (NPSR) A/T polymorphism (rs324981) and childhood maltreatment (CTQ) as well as an interactive G × E effect of these predictors on anxiety sensitivity (AS). Carriers of the more active NPSR T/T genotype or subjects with a high number of maltreatment experiences in childhood, respectively, reported increased AS, with the interaction of these factors further increasing AS.

These results are in line with the more active T allele being associated with panic disorder (Okamura et al. 2007; Donner et al. 2010; Domschke et al. 2011), increased AS and physiological stress responses (Domschke et al. 2011). The present results furthermore underline the importance of childhood maltreatment experiences in the pathogenesis of AS and anxiety disorders (Brown and Harris 1993; Stein et al. 1996; Bandelow et al. 2002; Scher and Stein 2003; Klauke et al. 2010). The presently observed interactive effect of NPSR gene variation and childhood trauma might be interpreted in the light of a study reporting intracerebroventricular or paraventricular nuclear administration of neuropeptide S to significantly increase plasma ACTH and corticosterone (Smith et al. 2006; Zhu et al. 2010). Thus, the more active NPSR rs324981 T allele might enhance the stressful influence of adverse life events by precipitating the endocrine response to stress. Also, NPSR mRNA has been demonstrated to be abundantly expressed in brain structures involved in learning and memory such as parahippocampal regions (subiculum, presubiculum, postsubiculum and parasubiculum), the lateral entorhinal cortex and the retrosplenial agranular cortex in rats and mice (Xu et al. 2007; Clark et al. 2011) and has been shown to facilitate long-term memory (Okamura et al. 2011). In this respect, memory contents related to, e.g., childhood trauma might be particularly retained by carriers of the more active NPSR rs324981 T allele. Furthermore, the more active NPSR T allele might confer an increased risk of anxiety in interaction with emotionally threatening experiences as it has been reported to be associated with a distorted cortico-limbic activation in healthy probands and patients with panic disorder, respectively, during processing of anxiety-relevant emotional stimuli (Dannlowski et al. 2011; Domschke et al. 2011). Also, increased activity in the rostral dorsomedial prefrontal cortex (dmPFC), an area supporting conscious appraisal of threat stimuli, was related to the NPSR T allele in a classic aversive conditioning paradigm in healthy participants (Raczka et al. 2010). Finally, the G × E effect of NPSR

### Table II. Results of the MIMIC analyses with gender, genetic, environmental and G × E variables as covariates.

<table>
<thead>
<tr>
<th>ASI subdimensions</th>
<th>Concern about mental/cognitive incapacitation</th>
<th>Concern about physical sensations</th>
<th>Concern about heart/lung failure</th>
<th>Concern about loss of control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>P</td>
<td>β</td>
<td>P</td>
</tr>
<tr>
<td>Predictors/covariates</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gender</td>
<td>0.054</td>
<td>0.34</td>
<td>0.141</td>
<td>0.01**</td>
</tr>
<tr>
<td>NPSR (T/T)</td>
<td>0.100</td>
<td>0.07</td>
<td>0.074</td>
<td>0.16</td>
</tr>
<tr>
<td>CTQ</td>
<td>0.305</td>
<td>0.00**</td>
<td>0.205</td>
<td>0.01**</td>
</tr>
<tr>
<td>LTE</td>
<td>−0.003</td>
<td>0.96</td>
<td>−0.004</td>
<td>0.95</td>
</tr>
<tr>
<td>NPSR (T/T) x CTQ</td>
<td>0.178</td>
<td>0.02</td>
<td>0.232</td>
<td>0.00**</td>
</tr>
<tr>
<td>NPSR (T/T) x LTE</td>
<td>−0.013</td>
<td>0.85</td>
<td>0.001</td>
<td>0.98</td>
</tr>
</tbody>
</table>

ASI, Anxiety Sensitivity Index; CTQ, Childhood Trauma Questionnaire (sum score); LTE, List of Threatening Experiences (sum score); NPSR (T/T) = T/T vs. A/T and A/A genotypes; β, standardized regression coefficient; P, P value, significant (bold) at significance value of < 0.05. *P < 0.05; **P < 0.01.
gene variation and early traumatic experiences might be mediated by epigenetic processes as suggested previously for neuropeptide systems in general (Cushing and Kramer 2005).

Interestingly, structural equation modelling using a MIMIC analysis revealed differential G × E effects of temporally proximal and distal life events on specific AS subfactors: “concern about physical sensation” and “concern about mental/cognitive incapacitation” were influenced by NPSRT/T/T genotype and its interaction with childhood trauma (CTQ), whereas the acute somatic subfactor “concern about heart/lung failure” was significantly influenced by NPSR T/T genotype and its interaction with recent life events (LTE). These results are in line with a previous report of differential influences of genetic and environmental predictors on specific ASI subdimensions, with childhood maltreatment influencing the more cognitive, and genetic factors (5-HTT gene variation) impacting the more somatic factors of the ASI, respectively (Klauke et al. 2011). The present results extend this observation by differentiating between early and recent life stress regarding their impact on anxiety sensitivity: the finding of childhood trauma on the one hand particularly influencing more general, temporally stable cognitive subfactors of the ASI is supported by previous studies showing an influence of stressful life events in childhood on overall appraisal and coping styles in adulthood (Compas et al. 1988; Pynoos et al. 1999; Jackson and Warren 2000) and once more stresses the important role of cognitive factors in the etiology of anxiety disorders (Clark 1986). The present finding of recent life events on the other hand on concern about acute somatic symptoms (heart/lung failure) is in line with studies reporting an impact of temporally proximal stressful life events on somatic symptoms and anxiety in adolescents (Poikolainen et al. 1995), panic disorder (e.g., Faravelli 1985; for review see Klauke et al. 2010) as well as panic disorder with somatic symptoms or somatic illness such as angina, hypertension, respiratory illness, migraine headache, diabetes mellitus, etc. (Härter et al. 2003). Thus, the investigation of more precisely defined endophenotypes such as ASI subdimensions may be instrumental in disentangling differential influences of genetics, environmental factors and G × E in the etiology of anxiety and anxiety disorders.

As there are only very few studies investigating the interaction of genetic and environmental predictors in a G × E study approach in anxiety-related phenotypes, and to the best of our knowledge no study has been published so far particularly analyzing NPSR × E, the present results have to be considered preliminary and are in need of further replication. Also, although the present sample size of N = 475 is among the largest in the field of G × E in anxiety-related phenotypes, even larger samples will help to decrease the risk of type II errors, especially in consideration of the relatively small effects in genetic studies. Furthermore, with a mean age of 25.2 years the present sample is relatively young, so that confounding effects of genetic predisposition of mental disorders manifesting at a later age cannot be excluded. The retrospective assessment of life events might have been flawed by recall bias or false answers (cf. Henry et al. 1994), and the relatively low average sum scores of ASI, CTQ, and LTE might indicate super-normality of the present sample and therefore does not allow for generalization of the results to other populations. Finally, the present results were not corrected for the possibly confounding influence of other genetic variants potentially contributing to a G × E effect on anxiety sensitivity.

Thus, further studies including larger samples and applying more complex models integrating additional mediating and moderating factors, G × G × E interactions as well as epigenetic analyses are warranted to consolidate the present results of an interactive effect of NPSR gene variation and stressful life events on anxiety sensitivity. These studies will contribute to further elucidation of the complex aetiology of anxiety and thereby hopefully to the development of more targeted therapeutic or even preventive strategies for anxiety disorders based on an individual risk profile including genetic and environmental markers.

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Statement of Interest

None to declare.

References


