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Neuropeptide S (NPS) receptor genotype modulates basolateral amygdala responsiveness to aversive stimuli

Running title: NPSR genotype modulates amygdala responsiveness

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Abstract

Recent studies point to a role of neuropeptide S (NPS) in the etiology of anxiety disorders. In animal models, NPS and its receptor (NPSR) were shown to be highly expressed in the amygdala, a central structure in the fear circuit, also known to be hyper-responsive in anxiety disorders. Recently, a functional polymorphism in the NPS receptor gene (rs324981 A/T) has been associated with panic disorder and anxiety sensitivity. However, the role of NPSR gene variation in the modulation of fear-related amygdala responsiveness remains to be clarified.

In 79 healthy subjects genotyped for NPSR rs324981, amygdala responses were assessed by means of fMRI. Participants were presented with fear-relevant faces in a robust emotion processing paradigm frequently used to study amygdala responsiveness.

We observed a strong association of NPSR T alleles with right amygdala responsiveness to fear-relevant faces. The association peak was located in the basolateral amygdala. Furthermore, responsiveness to aversive stimuli within this basolateral amygdala cluster predicted participant's self-reported harm avoidance but not depression level.

We conclude that NPSR genotype is associated with increased amygdala responsiveness to fear-relevant stimuli. Thereby, NPSR rs324981 apparently causes an indirect effect on anxiety related traits and potentially contributes to the pathogenesis of anxiety disorders by shaping fear-related limbic activity.

Keywords: Neuropeptide S, emotion, amygdala, anxiety, fMRI, imaging genetics

Introduction

Anxiety disorders are highly prevalent and debilitating psychiatric diseases with a moderate to high degree of heritability (Hettema, *et al* 2001). Understanding underlying molecular genetic pathomechanisms and their associated neurobiological abnormalities is a major goal of current research efforts. Neuropeptides have been suggested to play a crucial role in the pathogenesis of stress, arousal and anxiety. Particularly, the recently discovered neuropeptide S (NPS) and agonists at its receptor (NPSR) were reported to elicit anxiolytic effects in several studies employing rodent models (Xu, *et al* 2004; Rizzi, *et al* 2008; Leonard, *et al* 2008; Vitale, *et al* 2008) and thus constitute promising candidates for research on fear processing, anxiety and anxiety disorders (Reinscheid, *et al* 2005a; Okamura and Reinscheid, 2007; Pape, *et al* 2010).

While NPSR precursor mRNA is strongly expressed in the locus coeruleus as the major source of noradrenergic transmission in the brain, NPSR mRNA is widely expressed throughout the central nervous system, including the amygdaloid complex, as investigated in rodent models (cf. Reinscheid, *et al* 2005a). E.g., Jüngling *et al* described a cell-specific localization of NPSR expression in projection neurons of the amygdala, in particular in basolateral amygdala (BLA) principle neurons (Jüngling, *et al* 2008). Furthermore, BLA activity was reported to be dependent on a NPS-responsive circuitry (Meis, *et al* 2008). Consequently, intra-amygdala injections of NPS were shown to modulate fear-potentiated startle (Fendt, *et al* 2010, lateral amygdala) and anxiety-related behavior (Jüngling, *et al* 2008, basolateral amygdala). The BLA is the primary input site of the amygdaloid complex and receives wide projections from thalamic nuclei and sensory association cortices. While the central nucleus seems to be essential for the basic species-specific defensive responses associated with fear, the basolateral amygdala is apparently associated with encoding the threat value of a stimulus (Davis and Whalen, 2001). Taken

together, the NPS system appears to mediate specific effects on synaptic transmission to and within the basolateral amygdala, which is important for processing of fear (Pape, *et al* 2010).

In the NPSR gene, a single nucleotide polymorphism has recently been discovered (rs324981 A/T) that leads to an Asn/Ile exchange at position 107 (Asn¹⁰⁷Ile), with the T allele (¹⁰⁷Ile) increasing NPSR expression and NPS efficacy at NPSR about tenfold (Reinscheid, *et al* 2005b; Bernier, *et al* 2006). In two previous studies, the NPSR T allele has consistently been found to be associated with panic disorder, elevated anxiety sensitivity, and increased autonomic arousal (Okamura, *et al* 2007; Domschke, *et al* 2010b).

The neurobiological underpinnings of anxiety reaction in healthy subjects and anxiety disorders point to a pivotal role of the amygdala, and amygdala hyper-excitability is regarded as the main neural substrate in the etiology of these illnesses, since an overwhelming majority of neuroimaging studies reported that patients suffering from various anxiety disorders show potentiated amygdala responsiveness to aversive, fear-relevant stimuli (Etkin and Wager, 2007; Sehlmeier, *et al* 2009; Domschke and Dannlowski, 2010). Also in healthy controls, several studies reported associations between amygdala responsiveness and (non-pathological) anxiety related traits (Fakra, *et al* 2009; Sehlmeier, *et al* 2010; Baeken, *et al* 2010), particularly in the basolateral parts of the amygdala (Etkin, *et al* 2004).

Given the recent evidence regarding NPS in the etiology of anxiety disorders in humans, and the modulation of (basolateral) amygdala responsiveness already demonstrated in animal models, we sought to investigate whether NPSR rs324981 A/T also impacts human amygdala responsiveness to anxiety-relevant (fearful and angry) facial expressions as measured with functional magnetic resonance imaging (fMRI). We hypothesized that the risk (T) allele is associated with increased amygdala responsiveness to fear-relevant stimuli (angry and fearful

faces) presented in a robust paradigm, already employed in several previous imaging genetics studies.

Materials and Methods

Subjects. 79 right-handed healthy subjects of European ancestry participated in the present study as part of an ongoing project investigating the neurogenetics of emotion processing. All subjects were free from any life-time history of psychiatric disorders according to DSM-IV criteria (American Psychiatric Association, 1994), diagnosed with the SCID-I interview (Wittchen, *et al* 1997). Further exclusion criteria were any neurological abnormalities, history of seizures, head trauma or unconsciousness, intake of any psychotropic medication, and the usual MRI-contraindications. One male subject had to be excluded for anatomical abnormalities discovered in the structural MRI-Images (hydrocephalus). Six subjects were excluded for excessive head movement (> 2 mm or $> 2^\circ$). The TPQ harm avoidance scale was administered (Cloninger, 1987) and trait anxiety was assessed with the State-Trait Anxiety Inventory (STAI, trait version) (Laux, *et al* 1981). The Beck Depression Inventory (BDI) (Beck and Steer, 1987) was employed to measure current depression level. Table 1 lists sociodemographic and questionnaire data of study participants dependent on NPSR rs324981 genotype group. The study was approved by the Ethics Committee of the University of Münster. After complete description of the study to the participants, written informed consent was obtained. Participants received a financial compensation of 30 €.

Genotyping. Participants were genotyped for the functional NPSR rs324981 A/T (Asn¹⁰⁷Ile) polymorphism according to published protocols (Domschke, *et al* 2010b). DNA isolated from venous blood samples was amplified by PCR using the primers F: 5'-GAAGGAAAAAATTAAAAATGAACCTCCCCAGGATTTCAT and R: 5'-

TTCTACCCAGGAGAAAGCGGGCAGTTTGATGCA, resulting in an amplicon size of 353 bp. Standard PCR was carried out in a 20 µl volume containing 45–60 ng of genomic DNA, 10 pmol of each primer, 200 mM dNTPs, 0.4U Taq DNA Polymerase (Eppendorf, Hamburg, Germany), 50mM KCl, 1.5mM MgCl₂ and 10mM Tris-HCl (pH 8.4). After a 5 min denaturation, 35 cycles were carried out consisting of 30 s at 94 °C, 30 s at 66 °C and 60 s at 72 °C, followed by a final extension time of 10 min at 72 °C. Amplicons were digested with TasI (Fermentas, St Leon-Rot, Germany) (1 U), separated for 2 h on a 15% polyacrylamide gel and visualized by silver-staining. Hardy–Weinberg criterion, as calculated by the online program DeFinetti (<http://ihg.gsf.de/cgi-bin/hw/hwa1.pl>; Wienker TF and Strom TM), was fulfilled (exact test; $p=.48$).

fMRI methods. The experimental fMRI paradigm was a frequently used, robust paradigm for eliciting amygdala responsiveness, which has been employed in several previous imaging genetics studies (Hariri, *et al* 2002; Hariri, *et al* 2005; Pezawas, *et al* 2005; Meyer-Lindenberg, *et al* 2006; Zhou, *et al* 2008; Fakra, *et al* 2009). The paradigm consisted of 4 blocks of a face processing task alternating with 5 blocks of a sensorimotor control task. During the face processing task, participants viewed a trio of faces (all expressing either anger or fear) from the Ekman and Friesen (Ekman and Friesen, 1976) stimulus set and selected 1 of the 2 faces (bottom) that was identical to the target face (top). Each face-processing block consisted of 6 images, balanced for target gender and emotion (angry or fearful). During the sensorimotor control blocks, participants viewed a trio of geometric shapes (circles and ellipses) and selected 1 of the 2 shapes (bottom) that was identical to the target shape (top). Each sensorimotor control block consisted of 6 different shape trios. All blocks were preceded by an instruction (“Match faces” or “Match shapes” in German) that lasted 2 s. In the face-processing blocks, each of the 6 face trios was presented for 4 s with a variable interstimulus interval of 2 to 6 s (mean, 4 s), for a total block length of 48 s. In the sensorimotor control blocks, each of the 6 shape trios was presented

for 4 s with a fixed interstimulus interval of 2 s, for a total block length of 36 s. Total task time was 390 s. Participant performance (accuracy and reaction time) was recorded.

Participants held a fiber-optic response pad with two buttons in their right hand. Images were projected to the rear end of the scanner (Sharp XG-PC10XE with additional HF shielding). T2* functional data were acquired at a 3 T scanner (Gyrosan Intera 3T, Philips Medical Systems, Best, NL), using a single shot echoplanar sequence with parameters selected to minimize distortion in the region of central interest, while retaining adequate signal to noise ratio (S/N) and T2* sensitivity. Volumes consisting of 34 slices were acquired (matrix 64^2 , resolution $3.6 \times 3.6 \times 3.6$ mm; TR=2.1 s, TE=30 ms, FA=90°). The slices were tilted 25° from the AC/PC line in order to minimize drop out artifacts in the orbitofrontal and mediotemporal region.

Functional imaging data were realigned and unwarped, spatially normalized to standard MNI space (Montreal Neurological Institute) and smoothed (Gaussian kernel, 6 mm FWHM) using Statistical Parametric Mapping (SPM8, <http://www.fil.ion.ucl.ac.uk/spm>). Six subjects had to be excluded due to excessive head movement (exclusion criterion > 2 mm and/or 2°). Onsets and durations of the two experimental conditions (faces and shapes) were modeled with a canonical hemodynamic response function in the context of the general linear model (GLM) and the model was corrected for serial correlations. For each participant, one contrast image was generated in each individual fixed-effects 1st level analysis comparing activation in response to fear-relevant faces with the shapes baseline. The resulting contrast images were then entered into 2nd level random-effects group analyses.

We first analyzed whether the amygdalae were significantly activated by the task. Therefore, we used a small volume correction approach with a threshold of $p < .05$, family wise error (FWE) corrected for the amygdalae. The amygdala was defined according to (Tzourio-Mazoyer, *et al* 2002) and the amygdala mask was created by means of the WFU pickatlas

(Maldjian, *et al* 2003). In a second step, we tested our main hypothesis of amygdala modulation by NPSR genotype by regressing number of risk (T) alleles on amygdala responsiveness to fear-relevant facial expressions using the same anatomical mask and statistical threshold.

Finally, mean contrast values from the cluster that was significantly modulated by genotype were extracted for each participant and further processed with SPSS 15.0. We correlated these extracted amygdala activation values with trait anxiety and harm avoidance scores, performed post-hoc comparisons of the three genotype groups and tested for gender effects.

For exploratory reasons, a supplementary whole-brain analysis of NPSR rs324981 T allele effects on brain responsiveness was conducted at $p < .001$, uncorrected, with a cluster threshold of $k=20$ voxels (Table 2 for results). The anatomical labeling for the whole-brain data was performed by means of the AAL-Toolbox (Tzourio-Mazoyer, *et al* 2002), and the Brodmann areas (BA) were identified with the Talairach Daemon atlas (Lancaster, *et al* 2000).

Results

Behavioral results. The genotype groups did not differ regarding behavioral data, any sociodemographic variables, or questionnaire measures (see Table 1).

fMRI results. As in previous imaging genetics studies, the paradigm robustly activated the bilateral amygdala (right: $x=28$, $y=-2$, $z=-24$, $Z>8$, $p_{\text{FWE-corrected}} < .001$, cluster size $k=178$; left: $x=-30$, $y=0$, $z=-24$, $Z=7.12$, $p_{\text{FWE-corrected}} < .001$, cluster size $k=114$).

The regression analysis revealed a strong positive association of NPSR rs324981 T alleles and amygdala responsiveness to fearful/angry faces ($x=36$, $y=-2$, $z=-24$; $Z=3.91$, $p_{\text{uncorrected}}=.00004$; $p_{\text{FWE-corrected}}=.01$; $r=.44$, cluster size $k=6$ (Fig.1)). To determine the amygdala subregion, the SPM Anatomy toolbox Version 1.5 (Eickhoff, *et al* 2005) was employed.

According to the implemented probabilistic cytoarchitectonic maps (Amunts, *et al* 2005), the cluster was located in the basolateral amygdala. Mean contrast values for this cluster were extracted for each subject and further processed using SPSS 15.0 in order to compare the three genotype groups among each other and for correlation analyses.

For the between genotype groups comparison, we conducted an ANOVA on the cluster contrast values with genotype group as between-subjects factor. Paralleling the regression analysis, a strong group effect emerged, $F(1,71)=8.05$, $p=.001$. According to post-hoc Scheffe tests, AT and TT carriers showed significantly increased amygdala responsiveness compared to AA carriers ($p=.01$ and $p=.002$, respectively), whereas no significant difference between AT and TT carriers occurred ($p=.33$).

Responsiveness of the basolateral amygdala cluster correlated significantly with harm avoidance ($r=.37$, $p=.001$), but not trait anxiety ($r=.13$, $p=.27$). A multiple regression analysis with basolateral amygdala responsiveness and NPSR rs324981 T alleles as predictors of harm avoidance confirmed the strong association of amygdala responsiveness and harm avoidance ($\beta=.36$, $p=.005$), with no direct effect of T alleles ($\beta=.04$, $p=.75$), indicating an indirect effect on harm avoidance via amygdala responsiveness. Including the interaction term of amygdala responsiveness and NPSR rs324981 T alleles into the model did not produce any significant results for the interaction term and almost no changes to the other predictors. Furthermore, there was no association of amygdala responsiveness with depression levels as measured with BDI scores ($r=.09$, $p=.46$).

Effects of Gender: Given (contradictory) gender-specific results in the categorical association studies with panic disorder (Okamura, *et al* 2007; Domschke, *et al* 2010b), we conducted an exploratory correlation of the extracted basolateral amygdala contrast values and T alleles within each gender group separately. Our analysis revealed significant genotype effects for

each gender with a slightly higher association in women (men: $r = .36$, $p = .045$; women: $r = .49$, $p = .001$), in line with female-dominant effects reported in our previous study (Domschke, *et al* 2010b).

The whole-brain analysis yielded other structures in which neural activity during fear processing was positively associated with NPSR T alleles (see Table 2). There was no anatomical area showing a significant positive association with number of NPSR A alleles.

Discussion

In the present study, we demonstrated a robust effect of a recently described variant in the NPS receptor gene (NPSR rs324981 A/T) on amygdala responsiveness to fear-relevant facial expressions, with the more active T allele conferring increased right amygdala responsiveness to fearful/angry faces. This result is entirely in line with previous findings of the more active T allele to be associated with panic disorder, anxiety sensitivity and elevated autonomic arousal (Okamura and Reinscheid, 2007; Domschke, *et al* 2010b; Donner, *et al* 2010), while, quite counterintuitively, in the rodent model NPS has been shown to act as a potent anxiolytic (see introduction). In general, it has to be stated that pharmacological interventions during adulthood do not readily mimic genetically driven alterations during ontogeny. As with the serotonin transporter paradox (see Sibille and Lewis, 2006), also for NPS it could be speculated that high NPS levels in early stages of development might have a detrimental effect, while at later stages in life NPS might be beneficial with respect to anxiety states. A more specific possible explanation of the apparently contradictory directions of allelic association might be that anxiety is to a great extent conferred via an increased level of arousal (Blechert, *et al* 2007, Bouton, *et al* 2001), which in animal models has explicitly been found to be driven by increased NPS activity (Reinscheid, *et al* 2005a; Xu, *et al* 2004; Leonard, *et al* 2008; Rizzi, *et al* 2008). Increased

arousal as conferred by the more active T allele would furthermore be supported by reports of NPS to cause a significant stimulation of the hypothalamo-pituitary-adrenal (HPA) axis and concomitant increased arousal-like behavior in rats (Smith, *et al* 2006). Thus, the present finding of association of the more active T allele with anxiety-relevant brain activation patterns could be due to its arousal-increasing properties as suggested before (cf. Domschke, *et al* 2010b).

Numerous researchers have argued that the amygdala plays a central role in a neural circuit processing fear and anxiety and mediating arousal (Ledoux, 2000; Davis and Whalen, 2001; Sehlmeier, *et al* 2009). High amygdala responsivity to negative stimuli was shown to be associated with cognitive biases favoring processing of negative stimuli (Dannlowski, *et al* 2007a). The basolateral amygdala has been argued to represent the central input structure of the amygdaloid complex and is critically involved in the generation of affect (Davis and Whalen, 2001). Also in humans, neuroimaging studies have identified the right basolateral amygdala as being the main amygdala subarea associated with anxiety or hyperactivity in affective disorders (Etkin, *et al* 2004; Suslow, *et al* 2010). Interestingly, in the present study there was no association with depression level and amygdala responsiveness, potentially indicating an anxiety specific effect and not a general effect of negative emotions. Again, also this is in accordance with animal studies, where NPS agonists had no effect on immobility time in the tail suspension test (TST) suggesting no major antidepressant-like activity (Leonard, *et al* 2008).

Two recent studies have investigated NPSR rs324981 effects on brain activation patterns by fMRI: Domschke, *et al* reported significantly decreased activity in the anterior cingulate cortex, dorsolateral prefrontal and orbitofrontal cortex in response to fearful faces in patients with panic disorder carrying the NPSR rs324981 T risk allele, potentially reflecting a distorted cortico-limbic interaction during emotional processing (Domschke, *et al* 2010b). Using a classic aversive conditioning paradigm in healthy participants, Raczka, *et al* in turn reported the T allele to be

associated with increased activity in the rostral dorsomedial prefrontal cortex (dmPFC) evoked by the conditioned stimulus, an area that supports the explicit, conscious appraisal of threat stimuli (Raczka, *et al* 2010). However, in both studies explicit amygdala modulation by NPSR genotype has not been observed, potentially either due to a small sample size, a partially medicated patient sample or a ceiling effect of amygdala activation in patient with panic disorder (Domschke, *et al* 2010b) or the use of a conditioning paradigm (Raczka, *et al* 2010), which was not specifically designed to activate the amygdala but rather other structures of the fear circuit. In contrast, our present study employed a paradigm particularly designed for studying amygdala responsiveness to fear-relevant stimuli.

We detected an association of amygdala responsiveness and NPSR genotype only for the right amygdala. This might be due to the fact that overall there was also a somewhat stronger activation of the right amygdala by this specific task. Furthermore, at a more lenient threshold ($p < 0.05$, uncorrected) there was a small cluster demonstrating the same effect also within the left amygdala.

Additionally, we could demonstrate that the genetically modulated basolateral amygdala responsiveness to fear-relevant stimuli was directly associated with the participants' self-reported anxiety-related personality traits (harm avoidance), which is in line with previous neuroimaging studies (Fakra, *et al* 2009; Sehlmeier, *et al* 2010; Etkin, *et al* 2004; however, see Baeken, *et al* 2010). Albeit we did not find a significant association of trait anxiety with the responsiveness of the investigated basolateral amygdala cluster, it should be noted that at a more lenient threshold, such a positive association was also evident in our sample ($x=34$, $y=6$, $z=-20$, $Z=1.89$, $p_{\text{uncorrected}}=0.029$).

Our supplemental whole brain analysis yielded other anatomical areas modulated by NPSR genotype in the same direction as the amygdala. Albeit none of these clusters would

survive a rigorous alpha correction for the whole brain, some areas are of particular interest due to their widely reported involvement in emotion processing. Particularly, in line with the Domschke, *et al* finding, dorsolateral prefrontal cortex clusters (DLPFC) were observed at the uncorrected significance level, as well as right insular clusters, an orbitofrontal area and activity within the dorsal ACC. The OFC plays a role in the mediation of autonomic changes accompanying affective states produced in response to emotive stimuli or contexts, suggestive of a role for this region in the automatic regulation of emotional behavior (Phillips, *et al* 2003). The association of neural responses in these areas with T alleles might indicate an increased subjective experience of emotions and autonomic arousal during the processing of the angry and fearful expressions. On the other hand, the DLPFC and dorsal ACC were shown to be involved in executive control and emotion regulation. This might reflect a compensatory engagement in (healthy) risk allele carriers who experience the necessity to regulate their emotions more strongly than subjects with lower amygdala responsiveness.

Some limitations must be acknowledged. We have not genotyped our subjects for other potentially relevant polymorphisms which could modulate amygdala responsiveness, e.g., 5-HTTLPR, 5-HT_{1A} 1019C/G, or neuropeptide Y (NPY) among others (Dannlowski, *et al* 2007b; Dannlowski, *et al* 2008; Dannlowski, *et al* 2009; Dannlowski, *et al* 2010; Domschke, *et al* 2010a). The present effect size of $r=.44$ (equivalent to $d=.99$) is in the same range as reported effects of neuropeptide Y (Domschke, *et al* 2010a) and potentially even higher compared to 5-HTTLPR (Munafò, *et al* 2008). However, a direct comparison of different polymorphisms regarding effect sizes is not possible with our present data. The sample size was relatively small for a genetic association study but on the other hand it was large for an imaging genetics study, exceeding recommended sample sizes for this kind of analysis (Munafò, *et al* 2008). Nonetheless,

our sample size was particularly small for analysing subsamples stratified for gender and therefore these results should be treated with care.

In sum, our study provides further support of a strong role of neuropeptide S and its receptor in the genetic and neural underpinnings of anxiety and anxiety disorders. It provides a missing link between the results of animal studies, human genetic association studies and neuroimaging results and integrates these findings. Taken together, these findings might stimulate future studies involving the exploration of therapeutic agents targeting the NPS system in anxiety disorders.

Disclosures/ Conflict of Interests

Prof. Volker Arolt, M.D., Ph.D. is member of advisory boards and/or gave presentations for the following companies: Astra-Zeneca, Janssen-Organon, Lilly, Lundbeck, Servier, Pfizer, and Wyeth. He chairs the committee for the “Wyeth Research Award Depression and Anxiety”, now the DGPPN/Pfizer “Clinical Neuroscience Award”. Prof. Katharina Domschke, M.A., M.D. Ph.D., is on the speakers’ board of Pfizer, Lilly and Bristol-Myers Squibb and has received funding by Astra Zeneca. These affiliations have no relevance to the work covered in the manuscript. All other authors have no conflicts of interest to declare, financial or otherwise.

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Table 1

Task performance, sociodemographic, and affective characteristics of study participants dependent on NPSR rs324981 A/T genotype.

	AA (N=20)	AT (N=39)	TT (N=13)	p-value, according to χ^2 -test (df=2) or ANOVA ($F_{2,71}$)
Age	36.1 (10.6)	38.3 (10.1)	33.2 (8.4)	0.27
Sex (m/f)	7/13	19/20	5/8	0.56
Verbal IQ (MWT-B) ¹	114.4 (11.2)	119.9 (12.2)	120.8 (12.2)	0.20
Education years	16.0 (2.1)	15.7 (2.0)	15.2 (2.2)	0.58
% correct faces	99.5 (1.5)	99.5 (1.4)	99.0 (2.5)	0.66
% correct shapes	99.3 (1.7)	98.2 (2.2)	98.5 (2.2)	0.79
Mean RT faces (ms)	1050.1 (212.6)	1009.1 (124.2)	986.2 (191.4)	0.51
Mean RT shapes (ms)	855.7 (136.4)	833.4 (95.4)	810.1 (150.6)	0.57
STAI-trait ²	30.3 (4.7)	31.2 (6.5)	33.4 (5.4)	0.34
TPQ-HA ³	8.2 (5.0)	9.5 (3.8)	10.6 (4.3)	0.27
BDI ⁴	1.3 (1.6)	1.5 (1.7)	1.1 (1.6)	0.78

¹MWT-B, Mehrfachwahl-Wortschatz-Intelligenztest (Lehrl, 1995) ²STAI-trait, State-Trait

Anxiety Inventory (Laux, *et al* 1981); ³TPQ-HA, Tridimensional Personality Questionnaire, harm

avoidance scale (Cloninger, 1987); ⁴BDI, Beck Depression Inventory (Beck and Steer, 1987)

Table 2

Results of a whole brain regression analysis of NPSR rs324981 T alleles on neural responsiveness to fear-related facial expressions conducted at $p < .001$, uncorrected, $k = 20$ voxels.

Anatomical region	BA	Side	Cluster size	x	y	z	Z- score	p-value (uncorr.)
Middle Cingulate / dACC	24/32	R/L	247	2	8	42	4.71	<.00001
Precentral gyrus	6	R	162	42	-8	44	4.58	<.00001
Precentral gyrus	6	L	142	-32	-8	42	4.30	<.00001
IOG	18	R	77	24	-98	0	4.29	<.00001
Insula / STG	13/22	L	44	-44	-8	8	4.21	.00001
IFG, triangular part / MFG	46	L	50	-48	36	14	4.12	.00002
SOG / Cuneus	18/19	L	36	-6	-99	18	4.07	.00002
Middle Cingulate	24	R	57	10	-12	38	3.92	.00004
Amygdala	---	R	20	36	-2	-24	3.91	.00005
MFG	10	L	35	-28	54	18	3.83	.00006
Insula /IFG, orbital part	13/22/ 38/47	R	95	52	16	-8	3.83	.00006
MFG / IFG, opercular part	9	L	41	-42	18	38	3.82	.00007
IFG, triangular part	45	L	20	-34	26	12	3.60	.00016
Insula / Putamen / Hippocampus	13/21	R	53	40	-14	-8	3.58	.00017
Angular gyrus	39	L	20	-42	-48	30	3.52	.00022

Coordinates are given in MNI space. MFG, Middle frontal gyrus; IFG, inferior frontal gyrus; IOG, inferior occipital gyrus; SOG, superior occipital gyrus; SFG, superior frontal gyrus; BA, Brodmann area

Figure 1

NPSR rs324981 T risk alleles are positively associated with right basolateral amygdala responsiveness to fear relevant faces.

Left panel: Scatterplot depicting activity of the basolateral amygdala cluster (middle panel) dependent on genotype.

Middle panel: Coronal view ($y=0$) depicting amygdala responsiveness significantly modulated by genotype (at $p<.05$, FWE corrected for the bilateral amygdala).

Right panel: Scatter plot depicting a positive correlation ($r=0.37$, $p=.004$) of the amygdala cluster responsiveness and TPQ harm avoidance scores.

