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# Modulation of prefrontal functioning in attention systems by *NPSR1* gene variation

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ABSTRACT

Evidence has accumulated for a dysfunction of arousal and executive attention in anxiety. The neuropeptide S (NPS) system has been shown to play a pivotal role in the mediation of arousal and to be associated with anxiety/panic disorder. The present study aims at investigating the impact of functional neuropeptide S receptor (*NPSR1*) gene variation on neural attention patterns applying an imaging genetics approach.
In an event-related functional magnetic resonance imaging (fMRI) setting, 47 healthy subjects (f = 23) evenly pre-stratified for *NPSR1* rs324981 A/T genotype were investigated for brain activation patterns while performing the Attention Network Task (ANT), simultaneously probing alerting and executive control functions. Anxiety sensitivity was ascertained by the Anxiety Sensitivity Index (ASI).
In the alerting condition, *NPSR1* TT homozygotes showed higher activations in the right prefrontal cortex and the locus coeruleus region as compared to A allele carriers. In the executive control condition, TT homozygotes displayed increased activations in fronto-parietal regions. Genotype-driven activation differences in the prefrontal cortex correlated with anxiety sensitivity, in both the alerting and the executive control system.
The present results for the first time suggest *NPSR1* gene variation to be associated with alterations of prefrontal functioning in the attentional functions alerting and executive control partly modulated by anxiety sensitivity. These findings may aid in unraveling the neurobiological underpinnings of distorted arousal and attention in anxiety and thereby possibly in the biomarker-guided development of preventive/therapeutic strategies targeting attention processes in anxiety disorders.



## Introduction

Anxiety disorders are frequent and disabling disorders with a 12-month prevalence of 14% (Wittchen et al., 2011) and a high individual and socioeconomic burden (Murray et al., 2013). The complex pathogenesis of anxiety disorders is strongly influenced by genetic factors with heritability estimates ranging between 30 and 67% (Hettema et al., 2001). On a psychophysiological and neurocognitive level, hypervigilance and heightened physiological arousal as well as impaired attentional performance have been proposed as some of the key pathomechanisms of anxiety and panic disorder in particular (Craske and Simos, 2013; Doberenz et al., 2010; Eysenck, 1997; Eysenck et al., 2007; Howells et al., 2012; Meuret et al., 2011; Parente et al., 2005; for review see Geiger et al., 2014).

Attentional processes as measurable by the Attention Network Task (ANT; Fan et al., 2002) comprise the arousal-related 'alerting' system, the 'orienting' system and the 'executive control'/'conflict' system (Posner and Petersen, 1990). Anxiety as well as different anxiety disorders was found to be associated with neuropsychological alterations in the alerting and executive control systems; for example, trait anxiety was found to be related with executive attention skills, while state anxiety seemed to influence the efficiency of the alerting system (Pacheco-Unguetti et al., 2010; Pacheco-Unguetti et al., 2011). Anxiety in response to inhalation of carbon dioxide was reported to be associated with increased alerting (Garner et al., 2012).

Distinct neural activation patterns and related neurotransmitter systems have been proposed to underlie the respective attentional functions: the locus coeruleus (LC), right lateralized fronto-parietal regions and the thalamus mainly influenced by the norepinephrine

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system have been associated with the alerting system, while the anterior cingulate cortex, the dorsolateral prefrontal cortex (PFC) and the striatum have been linked to the executive control system and dopaminergic neurotransmission (Fan et al., 2005; Petersen and Posner, 2012; Posner and Petersen, 1990). A moderate to high heritability has been shown for the alerting ($h^2 = 0.18$) and the executive control ($h^2 = 0.89$) systems, but not for the orienting system ($h^2 = 0.0$) (Fan et al., 2001), rendering both alerting and executive attentional functions valid intermediate phenotypes of anxiety (cf. Domschke and Dannlowski, 2010). Alerting has previously been found to be associated with variation in the *ADRA2A*, *NET*, *MAO-A*, *GRIN2B* and *CACNA1C* genes, while executive control was reported to be related to *DAT1*, *DRD4*, *COMT*, *MAO-A*, *TPH2* and *DTNBP1* gene variation (e.g., Fossella et al., 2002; for review see Green et al., 2008).

Recently, converging evidence strongly implicated the neuropeptide S (NPS) system in the etiology of anxiety: In rodent models, NPS has been observed to exert an anxiolytic effect in several anxiety-behavior related tests, which, interestingly, was paralleled by robustly increased arousal (e.g., Rizzi et al., 2008; Wegener et al., 2012; Xu et al., 2004; for review see Okamura and Reinscheid, 2007; Pape et al., 2010; Reinscheid and Xu, 2005). In rats, NPS is primarily expressed in an area adjacent to the LC, while its cognate receptor (NPSR) has been reported to be widely expressed in various brain regions such as several ascending arousal pathways in the rat (Xu et al., 2007) as well as the murine orbital and frontal cortex (Clark et al., 2011). The human gene coding for the neuropeptide S receptor (*NPSR1*) on chromosome 7p14 contains an A/T single nucleotide polymorphism (rs324981) leading to an amino acid exchange (Asn107Ile), with the T allele (107Ile) conferring a 10-fold increased NPSR expression and NPS efficacy at the receptor (Reinscheid et al., 2005). The more active T allele and particularly the TT genotype were consistently found to be associated with increased fear ratings in a Pavlovian conditioning experiment (Raczka et al., 2010), heightened amygdala and PFC response to anxiety-related emotional stimuli (Dannlowski et al., 2011), enhanced response inhibition and increased error monitoring (Beste et al., 2013), increased autonomic arousal as reflected by accelerated heart rate and more intense symptom reports during a behavioral avoidance test (Domschke et al., 2011), elevated anxiety sensitivity (AS)—partly in interaction with negative life events (Domschke et al., 2011; Klauke et al., 2014), and finally—mostly in a female-specific fashion—panic disorder as a categorical disease entity (Domschke et al., 2011; Donner et al., 2010; Okamura et al., 2007). Given the suggested role of the NPS system in mediating a disturbed or hypersensitive arousal–anxiety relationship, the *NPSR1* gene constitutes a promising and yet to be investigated candidate in shaping anxiety-related attention systems.

Thus, in order to elucidate the impact of *NPSR1* gene variation on attention systems, in the present imaging genetic study neural activation patterns of alerting and executive control were studied dependent on *NPSR1* rs324981 genotype and AS in healthy probands using the ANT. It was hypothesized that the *NPSR1* TT risk genotype would be associated with both disturbed alerting and executive control systems: based on the functional neuroanatomy of the neuropeptide S system, we expected TT homozygotes to display a heightened alerting response reflected by stronger activation of the LC area and the right PFC, but with little effect on parietal processing. With regard to neural activation in the executive control condition, genotype-induced alterations were assumed to be localized predominantly within the PFC potentially reflecting a compensatorily enhanced neural response (cf. Beste et al., 2013). For both systems, correlations between brain activation and anxiety sensitivity were expected.

## Methods

### Subjects

Fourty-seven participants (f = 23, m = 24; mean age: 25.43 ± 2.7 years) were drawn from a large pool of healthy German subjects of Caucasian descent consecutively recruited at the Department of Psychiatry, University of Wuerzburg, Germany (Baumann et al., 2013; Domschke et al., 2011; Klauke et al., 2014). The present sample was pre-stratified according to *NPSR1* rs324981 genotype (AA: N = 16, AT: N = 16, TT: N = 15) (see below for genotyping and Table 1 for demographic and psychometric characteristics). Manifest or life-time history of mental axis I disorders was excluded by experienced clinical psychologists or psychiatrists using the Mini International Neuropsychiatric Interview (MINI) (Lecrubier et al., 1997). Pregnancy, severe medical conditions, and use of illegal drugs were further exclusion criteria. Anxiety sensitivity was ascertained using the German version of the Anxiety Sensitivity Index (ASI) comprising 16 items (Alpers and Pauli, 2001; Reiss et al., 1986). Right-handedness was ascertained using the Edinburgh Handedness Inventory (EHI; Oldfield, 1971). The study was approved of by the ethics committee of the University of Wuerzburg, Germany, and was conducted in accordance with the declaration of Helsinki in its latest version from 2008. Written informed consent was obtained from all subjects.

### Genotyping

Genotyping for the functional neuropeptide S receptor (*NPSR1*) A/T ($N^{107}I$) variant (rs324981) was performed according to published protocols (Domschke et al., 2011; Domschke et al., 2012). Genotypes were determined by investigators blinded for phenotypes and independently by two investigators. Testing for Hardy–Weinberg criteria was not applicable as participants included in the present study were pre-stratified according to genotype (see above).

### Paradigm

The Attention Network Task (ANT) by Fan et al. (2001) was implemented to study the efficiency and neural activation of the alerting network, the orienting network and the executive control network in a single task (Fan et al., 2005). In this task, five horizontally spaced arrows appeared below or above a fixation cross. Participants were asked to indicate the direction of the central arrow. The flanking context arrows pointed either in the same direction (congruent target condition) or in the opposite direction (incongruent target condition). On a random basis, three different cue-variations were presented in advance of the target appearance, defining the following cue conditions; (i) double cue: two asterisks appeared above and below the fixation cross indicating an imminent target appearance but without a hint toward the location of the target presentation, (ii) spatial cue: a single asterisk was presented either above or below the fixation cross indicating the spatial location of the next target and finally (iii) no cue. A trial started with fixation (400 ms) followed by either no cue, double cue or a spatial cue (150 ms), followed by fixation (400 ms) and the target (1050 ms). In order to ensure a variation between stimulus onset and image acquisition, null events were randomly presented in the course of the task. Null events did not represent an experimental condition and were thus not included into the statistical model.

Total trial duration for null events was 2000 ms, target events were 3000 ms long. Out of 256 trials there were 64 target events preceded by a double cue, 64 events preceded by a spatially informative cue, 64

**Table 1**
Demographic and psychometric characteristics of participants.

| Group | Total sample | *NPSR1* genotype groups (rs324981) | | | Statistics |
|---|---|---|---|---|---|
| | | TT | AT | AA | |
| N | 47 | 15 | 16 | 16 | |
| Sex, male/female | 24/23 | 11/4 | 7/9 | 6/10 | $\chi^2 = 4.5$ |
| Age, mean (SD) | 25.4 (2.7) | 24.8 (1.7) | 25.6 (2.9) | 25.8 (3.3) | $F(45) = 0.6$ |
| ASI, mean (SD) | 12.0 (7.1) | 13.9 (8.6) | 11.7 (7.2) | 10.7 (5.3) | $F(45) = 0.5$ |

SD = standard deviation; ASI = Anxiety Sensitivity Index.