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Original article

Neuropeptide S receptor gene variation and neural correlates of cognitive emotion regulation

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Abstract

The neuropeptide S (NPS) and its receptor NPSR have captured attention in the pathogenesis of anxiety disorders. Here, a functional polymorphism in the *NPSR1* gene has been linked to deviant cortico–limbic interactions in response to negative stimuli. While healthy T allele carriers exhibited increased amygdala and prefrontal cortex activity, panic disorder patients carrying the T risk allele displayed hypofrontality possibly reflecting insufficient prefrontal inhibition of limbic reactivity. In order to study multi-level effects of genotype and anxiety, prefrontal cortex activity during an emotional n-back task was measured in 66 volunteers genotyped for the *NPSR1* rs324981 A/T variant (AA homozygotes *vs*. T allele carriers) by means of functional near-infrared spectroscopy. For a high working memory load (3-back), T allele carriers showed a signal increase to negative pictures in the dorsolateral and medial prefrontal cortex while AA homozygotes displayed a signal decrease. Since groups did not differ on skin conductance level and behavioral parameters, this effect in the risk group in line with results from fMRI studies is speculated to represent an adaptive mechanism to compensate for presumably increased subcortical activity driven by an overactive NPS system. However, anxiety sensitivity correlated negatively with prefrontal activity in T allele carriers possibly suggesting a decompensation of the adaptive compensatory upregulation.

Key words: neuropeptide S; *NPSR1*; emotional working memory; anxiety; fNIRS

Introduction

In the last few years, the neuropeptide S (NPS) system has captured much attention as a promising novel pathomechanism of anxiety disorders (Tsuzuki *et al*., 2007). NPS administration in mice has been shown to produce anxiolytic-like effects in a battery of behavioral tests: NPS significantly increased the exploration of less protected or brighter areas in the open field (Xu *et al*., 2004; Jüngling *et al*., 2008), prolonged the time mice spent in the light zone of a light–dark box as well as within the open arms of the elevated plus maze (Xu *et al*., 2004; Jüngling *et al*., 2008), and dose-dependently reduced the number of marbles that were buried in the marble burying task (Xu *et al*., 2004; Vitale *et al*., 2008). In addition, NPS demonstrated arousal-promoting effects as indicated by an increase in locomotor activity and wakefulness (Xu *et al*., 2004). Pharmacologically, NPS binds to a G-protein-coupled receptor (NPSR) that stimulates intracellular calcium concentrations and cyclic adenosine monophosphate accumulation (Reinscheid *et al*., 2005). These NPS receptors are widely distributed in the central nervous system with highest expressions in the cortex, thalamus, hypothalamus and the amygdala (Xu *et al*., 2004; Reinscheid and Xu, 2005). The effects on synaptic transmission to and within the

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amygdala are of particular relevance since an increased glutamatergic synaptic transmission to intercalated GABAergic neurons in the amygdala has been identified to accompany the effects of NPS administration on mice behavior (Jüngling *et al.*, 2008).

While NPS is associated with anxiolytic-like effects in the rodent model, investigation of the NPS system in humans revealed divergent but nonetheless anxiety-related results: The human gene coding for the NPS receptor (*NPSR1*) on chromosome 7p14 contains an A/T single nucleotide polymorphism (SNP, rs324981) leading to an amino acid exchange (Asn^{107}Ile), with the T allele (^{107}Ile) conferring a 10-fold increased *NPSR1* expression and NPS efficacy at the receptor (Reinscheid *et al.*, 2005). The more active T allele was consistently found to be overrepresented in patients with panic disorder (Okamura *et al.*, 2007; Donner *et al.*, 2010; Domschke *et al.*, 2011). The T allele was also associated with increased autonomic arousal as evident in a heightened heart rate and more intense symptom reports during a behavioral avoidance test (Domschke *et al.*, 2011). Paralleling these findings, healthy T allele carriers showed significantly higher fear ratings in a Pavlovian conditioning experiment than AA homozygotes (Raczka *et al.*, 2010). The *NPSR1* T allele was further found to be associated with significantly elevated anxiety sensitivity (AS) — reflecting the tendency to cognitively (mis-)interpret anxiety-related bodily sensations (Reiss *et al.*, 1986) and constituting an intermediate phenotype and risk factor of pathological anxiety (Schmidt *et al.*, 1997, 1999, 2006) — in healthy probands in interaction with early life stress (Klauke *et al.*, 2012) as well as in patients with panic disorder (Domschke *et al.*, 2011).

Anxious individuals have been shown to be highly susceptible to emotionally loaded material (Bar-Haim *et al.*, 2007), resulting in a loss of concentration and impairments in executive functioning for the actual task, which has been linked to a reduced recruitment of top-down control mechanisms in the brain fear circuit (Bishop *et al.*, 2004). In imaging genetics approaches, *NPSR1* gene variation has been reported to drive a deviant cortico–limbic interaction potentially reflecting dysfunctional emotional processing. Healthy T risk allele carriers showed significantly increased amygdala activation along with increased dorsolateral prefrontal cortex (dlPFC), orbitofrontal cortex (OFC) and dorsal anterior cingulate (ACC) activity when passively watching fearful face stimuli. This increased prefrontal activation was suggested to represent a compensatory increased top-down regulation of amygdala activity evoked by negative emotional stimuli (Dannlowski *et al.*, 2011). Conversely, in a sample of panic disorder patients investigated with a similar task of passive emotion perception, the *NPSR1* T allele group showed decreased prefrontal cortex activity which was discussed as insufficient prefrontal inhibition of limbic activity in clinically manifest pathological anxiety (Domschke *et al.*, 2011).

To explicitly study multi-level effects of genotype and anxiety levels on cognitive emotion regulation, this study investigated healthy volunteers for their response to an emotional n-back task depending on the functional *NPSR1* A/T SNP (rs324981) by means of functional near-infrared spectroscopy (fNIRS), skin conductance level (SCL) and behavioral data. Based on the findings reviewed above, negative pictures were hypothesized to induce an increased prefrontal recruitment detectable with fNIRS in carriers of the more active T risk allele. On the other hand, increased AS, as an intermediate phenotype of pathological anxiety, was hypothesized to lead to a decompensation of this adaptive prefrontal upregulation in *NPSR1* T risk allele carriers as expressed by lower prefrontal recruitment during the processing of negative emotional stimuli.

Materials and methods

Participants

Sixty-six healthy Caucasian volunteers (female = 33, male = 33; mean age = 25.36 ± 4.8; years of education = 12.91 ± 0.5) participated in this study. They were recruited through online advertisements and screened for current mental health using the Mini International Neuropsychiatric Interview (Sheehan *et al.*, 1998) and for right handedness using the Edinburgh Handedness Inventory (Oldfield, 1971). In order to assess *NPSR1* genotype group differences on state and trait measurements of anxiety, the state version of the State-Trait-Anxiety Inventory (STAI; Laux *et al.*, 1981) and the Anxiety Sensitivity Index (ASI; Reiss *et al.*, 1986) were administered (STAI state anxiety = 32.23 ± 6; ASI = 13.42 ± 8.5). All participants signed written informed consent before taking part in the experiment and were reimbursed with 15 Euro. The study was approved by the Ethics committee of the University of Würzburg, Germany, and was conducted in accordance with the declaration of Helsinki in its latest version from 2008.

Genotyping

Genotyping of the functional *NPSR1* rs324981 A/T (Asn107Ile) polymorphism was performed according to published protocols (e.g. Bishop, 2009; Domschke *et al.*, 2011, 2012). In brief, DNA isolated from venous blood samples was amplified by PCR using the primers F: 5′-GAA GGA AAA AAA TTA AAA ATG AAC CTC CCC AGG ATT TCAT and R: 5′-TTC TAC CCA GGA GAA AGC GGG CAG TTT GAT GCA, resulting in an amplicon size of 353 bp. Standard PCR was carried out in a 20-ml volume containing 45–60 ng of genomic DNA, 10 pmol of each primer, 200 mM dNTPs, 0.4U Taq DNA Polymerase (Eppendorf, Hamburg, Germany), 50 mM KCl, 1.5 mM $MgCl_2$ and 10 mM 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. Due to genotyping failure in two probands, a sample of $N = 64$ remained for further analyses. Hardy–Weinberg criteria, as calculated by the online program DeFinetti (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl; Wienker TF and Strom TM), were fulfilled for genotype distribution (AA = 28, AT = 30, TT = 6, $P = 0.78$). For further analyses, *NPSR1* genotypes were grouped according to functionality and on the basis of previous studies assuming a dominant role for the T risk allele (AA *vs* AT/TT; Raczka *et al.*, 2010; Domschke *et al.*, 2011). The groups are further referred to as AA homozygotes on the one hand and T allele carriers for participants with at least one T allele (AT/TT genotype carriers) on the other hand.

Emotional n-back task

The task consisted of 90 colored photographs derived from the Emotional Picture Set (EmoPicS; Wessa *et al.*, 2010). Based upon the normative data provided for the EmoPicS database, they were selected according to their valence and arousal in order to group (a) 30 pleasant and (b) 30 unpleasant pictures with moderately high arousal and (c) 30 neutral pictures inducing only little to no arousal. Pleasant pictures depicted athletic activities, children

and couples in love (excluding erotic scenes). Unpleasant pictures depicted war scenarios showing injured or crying people, and neutral pictures mostly depicted people reading or walking without any emotional expression. All pictures illustrated people excluding merely sheer artificial or naturalistic content. Pictures were presented in nine blocks counterbalanced by three different working memory load manipulations namely 1-back, 2-back and 3-back. Each emotional category was thus presented once in all three n-back levels. The sequential arrangement of blocks was pseudorandomized in three different versions to prevent learning effects and habituation to the emotional picture content. Versions were counterbalanced across participants. Each block had a duration of 60 s and consisted of 30 pictures of which six were target trials. Pictures were presented for 500 ms followed by an inter-trial interval of 1500 ms depicting a black screen. The n-back level of each block was announced by an instruction slide and was started individually by the participant. Blocks were separated by a resting period of 30 s in which participants were instructed to relax.

To become familiar with the task, participants practiced each n-back level beforehand with pictures, which were not selected for the actual task. They were instructed to respond as fast and accurate as possible by button presses, irrespective of the emotional picture content. After the experiment, all participants evaluated the pictures regarding valence and arousal on two Likert scales ranging from 1 for 'very unpleasant' to 9 for 'very pleasant' and 1 for 'no arousal' to 9 for 'high arousal'.

Skin conductance level

SCL was measured by using two Ag/AgCl electrodes which were attached to the hypothenar eminence of the left hand. Recording was performed via the Vision recorder software (Brain Products GmbH, Munich, Germany), which operates with a sampling rate of 1000 Hz. Data were offline low-pass filtered at 12 Hz to correct for signal drifts. Each block was further baseline corrected 500 ms before block onset. Due to the response latency of the SCL, signal blocks were analysed 4 s after trigger onset. Mean activity of the resulting 56 s segments was calculated.

Functional near-infrared spectroscopy

Prefrontal cortex activation was measured by means of fNIRS, a non-invasive optical imaging technique which is explained in detail elsewhere (Obrig and Villringer, 2003). In brief, light from the near-infrared spectrum penetrating biological tissue is inducted to the skull by light emitters and gets partly absorbed in depth up to 2.5 cm of the cortex (Hoshi *et al.*, 2005) by oxygenated (O_2Hb) and deoxygenated (HHb) hemoglobin. The amount of reflected light at the surface can be detected, providing thus cortical concentration changes of O_2Hb and HHb. With regard to neurovascular coupling, neural activation is associated with increasing O_2Hb and decreasing HHb theoretically correlating perfectly negative (Cui *et al.*, 2010).

Hemoglobin concentration changes were measured with the continuous wave system ETG 4000 (Hitachi Medical Co., Tokyo, Japan) operating with two different wavelengths (650 ± 20 and 830 ± 20 nm). In order to cover the whole prefrontal cortex a 52-channel array consisting of 17 light emitters and 16 photo detectors was used. The middle detector in the lowest row was positioned on Fpz according to the 10-20 EEG system (Jasper, 1958), the lateral optodes extended approximately to T3 and T4. The interoptode distance was set to 3 cm. Data were recorded with a sampling rate of 10 Hz.

Data analysis

Preprocessing and statistical analysis were performed by using Matlab (2009a, The MathWorks Inc., MA, USA), Vision Analyzer 2.0 (Brain Products GmbH) and SPSS version 21 (IBM SPSS Statistics, Munich, Germany). On the behavioral level, accuracy was calculated as the ratio of hits and correct rejections to total number of trials (cf. Grimm *et al.*, 2012). Moreover, mean reaction times on hits were investigated. These parameters and SCL were analysed by repeated measurements (ANOVA) with working memory load (1-, 2-, 3-back) and emotion (positive, neutral, negative pictures) as within-subject factors and group (*NPSR1* genotype AA, T) as between-subject factor. Significant interaction effects were further elucidated by post-hoc Student's *t* tests at a significance level of $P < 0.05$ (two-tailed). In addition, *t*-contrasts were referred as Pearson's correlation coefficients (r_{con}) in order to provide an effect size with $r_{con} > 0.5$ characterizing large effects (e.g. Rosnow and Rosenthal, 2005). Non-sphericity was considered by applying the Greenhouse Geisser correction.

Based on the assumption that O_2Hb and HHb should be negatively correlated, a correlation-based signal improvement algorithm developed by Cui *et al.* (2010) was applied to the fNIRS data resulting in one integrated signal of both chromophores per channel (for previous studies using this algorithm please refer to e.g. Müller *et al.*, 2014; Tupak *et al.*, 2014). These signal changes were processed by applying a low-pass filter of 0.5 Hz and a cosine filter correcting for low-frequency signal drifts. The resulting nine segments had duration of 50 s starting 10 s after block onset. They were baseline corrected by using the time window of 5–4.5 s before block onset which represents the inter block resting period before participants were instructed with the following n-back condition. In order to analyse working memory load by emotion effects five regions of interest were defined (ROIs, see Figure 1): right dlPFC (channels 4, 14, 15, 25), left dlPFC (7, 17, 18, 28), right ventrolateral prefrontal cortex (vlPFC: 35, 45, 46), left vlPFC (39, 49, 50) and the medial prefrontal cortex (mPFC: 16, 26, 27, 37). The ROIs were chosen according to probabilistic registration methods (Tsuzuki *et al.*, 2007) and practical considerations. The dlPFC channels cover the middle frontal gyrus, the vlPFC channels the inferior frontal gyrus. The mPFC channels comprise the closest channels in the vicinity of the interhemispheric fissure above the medial PFC. According to the behavioral data analyses, ROIs were statistically evaluated by repeated measurements ANOVA with working memory load (1-, 2-, 3-back), emotion (positive, neutral, negative) and hemisphere (right, left) as within-subject factors and group (*NPSR1* genotype AA, T) as between-subject factor. fNIRS results were further correlated with measurements of anxiety depending on *NPSR1* genotype by calculating Pearson's (ASI) and Spearman's (STAI) correlation coefficients. To keep the amount of correlations as low as possible, only positive (mean positive – mean neutral) and negative picture blocks (mean negative – mean neutral) were analysed. Correlations were further evaluated concerning significant ($P_{one\text{-}tailed} < 0.05$) group differences by using the Fisher *r*-to-z transformation.

Results

Picture ratings

As expected, ANOVA revealed a main effect of valence [$F(1.8,113.1) = 763.56$, $P < 0.001$], with significant differences between all picture categories (positive > neutral > negative)

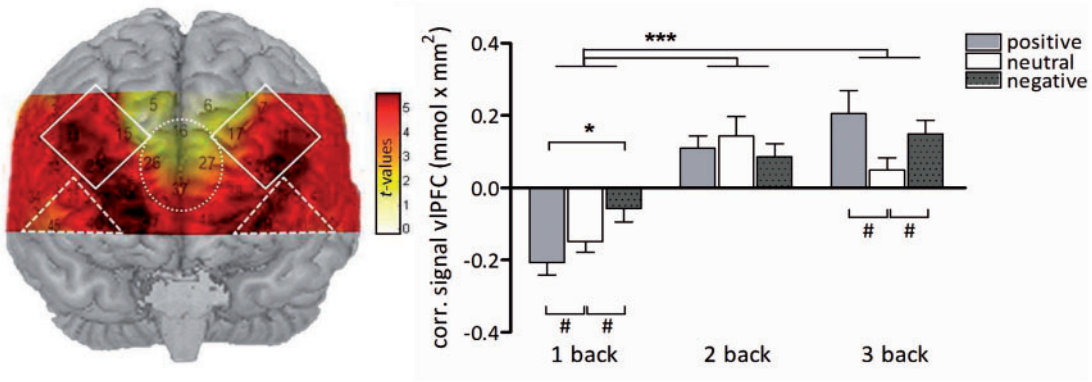

Fig. 1. Left: T-map superimposed on a standard brain showing the signal increase with increasing task demands (3-back *vs* 1-back, FDR corrected). Geometrical figures depict the ROI: dlPFC (rhombs), vlPFC (triangles), mPFC (oval). Right: Task-evoked corrected signal changes for the vlPFC in the whole group of $N = 66$. Asterisks indicate significant differences concerning a *P*-value which is either uncorrected ($^{\#}P < 0.05$) or corrected for multiple comparisons ($^{*}P_{corr} < 0.006$). The significant main effect of condition is indicated by $^{***}P < 0.001$.

[$t(64) \geq 18.8$, $P < 0.001$, $r_{con} \geq 0.92$]. Arousal also revealed a significant main effect [$F(2,128) = 206.65$, $P < 0.001$], with positive and negative pictures showing an equally high arousal [$t(64) = 1.78$, $P > 0.05$], while both significantly differed from the neutral picture category [$t(64) \geq 16.89$, $P < 0.001$, $r_{con} \geq 0.82$]. *NPSR1* genotype group did not reveal significant main or interaction effects.

Behavioral results

ANOVA revealed a main effect of condition [$F(1.4,87.2) = 132.52$, $P < 0.001$] and a significant condition × emotion interaction [$F(3,185.9) = 2.79$, $P < 0.05$]. To further elucidate this interaction, accuracy scores depending on emotional category were compared on every n-back level resulting in significantly higher accuracy scores for positive *vs* neutral pictures in the 3-back condition [$t(63) = 2.28$, $P < 0.05$, $r_{con} = 0.28$]. *NPSR1* genotype group did not reveal significant main or interaction effects.

Reaction times revealed a main effect of condition [$F(1.7,104.4) = 238.5$, $P < 0.001$], i.e. the more difficult the task the longer the reaction times [3-back > 2-back: $t(63) = 18.7$, $P < 0.001$; 2-back > 1-back: $t(63) = 13$, $P < 0.001$; $r_{con} > 0.85$]. Neither emotion nor genotype group showed significant main or interaction effects.

SCL results

Six participants were excluded as non-responders; data of two other participants were lost due to a technical problem during recording. ANOVA revealed significant main effects of condition [$F(1.8,95.1) = 6.88$, $P < 0.05$] and emotion [$F(2,108) = 6.11$, $P < 0.05$] and a marginally significant condition × emotion interaction [$F(1.7,91.3) = 3.17$, $P < 0.1$]. Medium and high working memory load were associated with an increased SCL [dependent *t*-test 1-back *vs* 2-back: $t(55) = -3$, $r_{con} = 0.38$; 1-back *vs* 3-back: $t(55) = -3.27$, $r_{con} = 0.4$; $P < 0.01$], and positive picture blocks evoked higher responses than neutral and negative blocks [positive *vs* neutral: $t(55) = 3.29$, $r_{con} = 0.41$; positive *vs* negative: $t(55) = 2.99$, $r_{con} = 0.37$; $P < 0.01$]. Positive pictures evoked a higher SCL than neutral pictures in the 1-back condition and also a higher SCL than negative pictures in the 2-back condition [$t(55) \leq 4.17$, $r_{con} \leq 0.49$, $P \leq 0.005$, Bonferroni corrected]. *NPSR1* genotype group-dependent analysis did not result in significant interactions.

fNIRS results

Whole group results (N = 66)

For the dlPFC, working memory load revealed a significant main effect [$F(1.8,112.2) = 12$, $P < 0.001$] which manifested in a linear signal increase from 1- via 2- to 3-back [linear trend test for condition: $F(1,63) = 18.17$, $P < 0.001$], and was more pronounced in the right than the left hemisphere [main effect of hemisphere: $F(1,63) = 10.96$, $P < 0.001$]. For the mPFC, condition exerted a main effect as well [$F(2,130) = 6.83$, $P < 0.01$], again showing a linear signal increase with difficulty [$F(1,65) = 10.95$, $P < 0.01$]. The working memory-related main effect was also present in both vlPFCs [$F(2,130) = 31.43$, $P < 0.001$], again with marginally higher values for the right hemisphere [main effect for hemisphere: $F(1,65) = 5.72$, $P < 0.05$]. In addition, a significant interaction between condition and emotion was discerned [$F(3,194.7) = 4.66$, $P < 0.01$] showing negative pictures to evoke a higher fNIRS signal than positive pictures at the 1-back level [paired sample *t*-test: $t(65) = 3.44$, $P_{corr} < 0.006$, $r_{con} = 0.39$, see Figure 1].

Results stratified for NPSR1 genotype

Due to genotyping failure in two participants, a sample of $N = 64$ remained for further analyses which however did not affect the results of the whole sample. Genotype groups (AA = 28 *vs* T = 36) did not differ in terms of sex, age, level of education and measures of anxiety (Table 1).

The interaction between working memory load and emotion revealed significant *NPSR1* genotype group differences for the dlPFC [$F(4,248) = 3.32$, $P < 0.05$] and the mPFC ROI [$F(4,248) = 5.2$, $P < 0.001$]. In order to disentangle the condition × emotion × hemisphere by *NPSR1* genotype group interaction for the dlPFC, post-hoc *t*-tests were performed and revealed significant group differences for the condition × emotion interaction in the left hemisphere. Here, the interaction with genotype group mainly consisted of a different activation pattern for positive and negative pictures in the 3-back condition. AA homozygotes displayed a higher fNIRS signal for positive pictures than T allele carriers [independent *t*-test: $t(62) = 2.22$, $P < 0.05$, $r_{con} = 0.27$], while T allele carriers showed a higher signal to negative pictures than AA homozygotes [$t(62) = 2.45$, $P < 0.05$, $r_{con} = 0.3$]. A comparable reciprocal activation pattern was evident in the mPFC: again, AA homozygotes showed a marginal signal increase for positive pictures along with increasing working memory load [3-back working memory load: $t(62) = 1.89$, $P < 0.1$, $r_{con} = 0.23$], whereas T allele carriers showed a signal increase

Table 1. Sample characteristics

	All	*NPSR1* AA	*NPSR1* T	P[a]
Sex (m/f)	33/31	15/13	18/18	0.806
Age	25.36 ± 4.8	25.5 ± 5.7	25.25 ± 4.2	0.839
Education (years)	12.91 ± 0.5	13	12.83 ± 0.7	0.211
ASI	13.37 ± 8.6	11.39 ± 7.8	14.92 ± 9	0.105
STAI state	32 ± 5.9	32.11 ± 5.9	31.92 ± 6	0.900

ASI, Anxiety Sensitivity Index; STAI state, state version of the State Trait Anxiety Index. Calculated are means and SEM.

[a]P-values indicate between-group differences as calculated by independent Student's *t* tests or Chi-square test

for negative pictures with increasing difficulty [3-back working memory load: $t(62) = 2$, $P \leq 0.05$, $r_{con} = 0.25$; Figure 2]. There was no group difference in response to neutral pictures, neither in the left dlPFC nor the mPFC ROI [$t(62) \leq 1.2$, $P < 0.05$].

The two ROIs for which significant interactions with *NPSR1* genotype were found were further analysed for correlations with ASI and STAI anxiety scores in both groups. Here, in T-risk allele carriers, but not in AA homozygotes, AS was significantly related to the fNIRS signal in the mPFC for negative ($r = -0.45$, $P < 0.01$) as well as for positive ($r = -0.35$, $P < 0.05$) pictures, in that *NPSR1* T allele carriers with high ASI scores showed significantly less mPFC activation. Accordingly, the ASI score was by trend inversely correlated with left dlPFC activation in response to negative pictures in T allele carriers only ($r = -0.31$, $P = 0.062$). All correlation coefficients significantly differed between groups ($z \geq 1.68$, $P < 0.05$) indicating a specific relationship between prefrontal activation and AS for the T allele group (Figure 3). In addition, in T allele carriers, but not in AA homozygotes, STAI state anxiety revealed an inverse correlation with mPFC ($r = -0.41$, $P < 0.05$) and left dlPFC activation ($r = -0.41$, $P < 0.05$) in response to negative pictures. Due to the fact that ASI scores and STAI state anxiety were significantly correlated, we conducted a partial correlation between STAI state anxiety and ROI activity while controlling for the ASI effect. This analysis confirmed the aforementioned results (mPFC: $r_{part} = -0.47$, dlPFC: $r_{part} = -0.52$, $P < 0.005$). However, correlation coefficients did not significantly differ between groups ($z \leq 1.01$, $P > 0.1$) emphasizing a specific relationship between AS and not STAI state anxiety and prefrontal activity in the T allele group.

Discussion

This study investigated the effects of *NPSR1* gene variation on emotional working memory by means of an emotional n-back task consisting of three working memory load conditions (1-, 2- and 3-back) and three picture categories (positive, neutral and negative pictures). While genotype groups (AA homozygotes *vs* T allele carriers) did not differ with regard to behavioral parameters such as accuracy and reaction times or skin conductance, the analyses of hemoglobin concentration changes in the prefrontal cortex measured with fNIRS revealed genotype-specific results in the mPFC and the left dlPFC: In the high working memory load condition (3-back), T allele carriers showed a signal increase in response to negative pictures and a signal decrease in response to positive pictures, while AA homozygotes displayed a reciprocal pattern. When additionally considering AS, a high ASI was associated with significantly decreased mPFC and left dlPFC activation in T allele carriers.

From previous studies it is known that the ability to control and modulate emotional responses depends on a cortical top-down modulation of the limbic system. For instance, Hariri *et al.* (2000) reported an inverse relationship between the prefrontal cortex and the amygdala in conscious semantic processing of emotional stimuli with the PFC exerting a modulating effect on emotional experience. In this study, *NPSR1* T-risk allele carriers showed significantly increased prefrontal activation (mPFC, dlPFC) in response to negative pictures in the highest working memory load condition, demanding utmost cognitive control. Given converging evidence for the more active *NPSR1* T allele to constitute a risk factor for panic disorder, to be associated with increased autonomic arousal and heightened fear conditioning (Okamura *et al.*, 2007; Donner *et al.*, 2010; Raczka *et al.*, 2010; Domschke *et al.*, 2011) and to drive higher amygdala activation along with increased dlPFC, OFC and dorsal ACC activity in response to fearful faces in healthy probands (Dannlowski *et al.*, 2011), the presently observed higher prefrontal engagement in response to negative emotional stimuli in T allele carriers may be interpreted as an adaptive compensatory engagement counterbalancing a presumably increased subcortical activity as conferred by an overactive NPS system. According to Dannlowski *et al.* (2011), the increased prefrontal activity might either be associated with an increased subjective experience of negative emotions or reflect an increased emotion regulation to cope with the requirements of the working memory task. Since the interaction between emotion and cognition can be considered as a competition for attentional resources (Vytal *et al.*, 2012), negative stimuli might have captured more attention than positive ones, probably on the basis of a threat-related attentional bias in anxious individuals (Bishop *et al.*, 2004; Bar-Haim *et al.*, 2007). However, in this study this was not evidenced on the behavioral level since accuracy reached a score of at least 93% even in the 3-back condition suggesting a ceiling effect. Neurobiologically, high arousal has been referred to an increased activity in both the amygdala and the dlPFC during an emotional working memory task (Perlstein *et al.*, 2002). Orientation toward threat associated stimuli in *NPSR1* T allele carriers in this study is also supported by the T allele carriers' tendency to over-interpret the harmfulness of aversive events and increased harm avoidance (Raczka *et al.*, 2010; Domschke *et al.*, 2011). In contrast, *NPSR1* AA homozygotes, i.e. non-risk allele carriers, displayed decreased prefrontal activation in response to negative and increased activation in response to positive pictures in the 3-back working memory load condition, which is in line with healthy participants exhibiting the same pattern in emotional word n-back tasks (Grimm *et al.*, 2012; Kopf *et al.*, 2013) as well as in an emotional picture detection task (Perlstein *et al.*, 2002). In accordance with Kopf *et al.* (2013), who found decreased prefrontal cortex activity on negatively valenced and increased activity on positively valenced word stimuli for the 2-back and 3-back conditions, the observed activation patterns in response to positive and negative pictures strongly point to a valence effect not confounded by arousal, as in this study positive and negative pictures were selected to achieve a comparable level of moderate arousal. Neutral pictures, which did not require the regulation of emotions but rather require mere working memory demands, did not result in significant group differences neither in the dlFPC nor in the mPFC. This supports the notion that differences observed in tasks with valenced pictures, which did show group differences for prefrontal activity, indeed are due to emotion regulation and not mere working memory processes. While the existing literature primarily focuse on top-down modulation of negative or

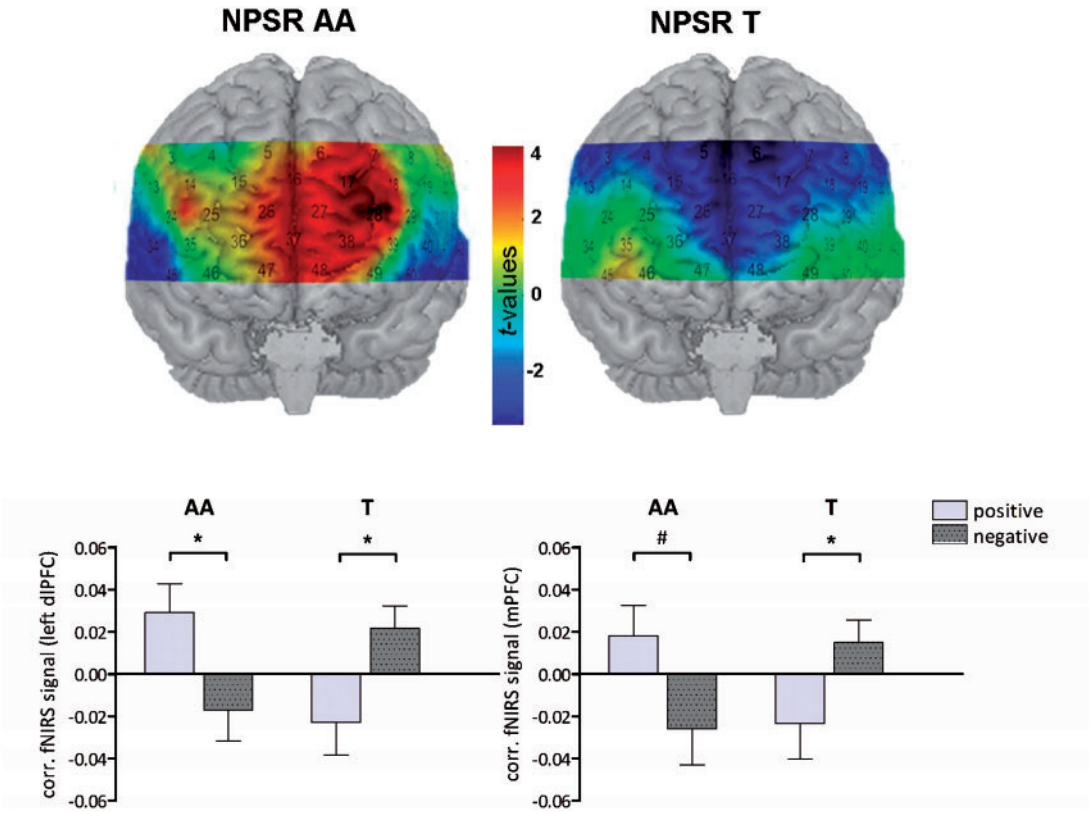

Fig. 2. Above: within-group fNIRS signals for prefrontal activation during the 3-back working memory load for positive *vs* negative pictures stratified for *NPSR1* genotype. While AA homozygotes (left) showed an increased fNIRS signal in regions covering the mPFC and left dlPFC, T allele carriers (right) showed deactivations in these areas. Depicted are *t*-values for all 52 channels ($P_{uncorr.} \leq 0.05$). Below: Corrected fNIRS signal changes in the left dlPFC (left) and mPFC (right) for positive and negative pictures (3-back condition) showing a significant interaction with *NPSR1* genotype (AA *vs* T). While in AA homozygotes the fNIRS signal was increased for positive pictures, T allele carriers responded to negative pictures with a signal increase. Depicted are means and SEM. Asterisks indicate significant differences ($^{*}P < 0.05$), the rhomb mark indicates a trendwise significant result ($^{\#}P < 0.1$).

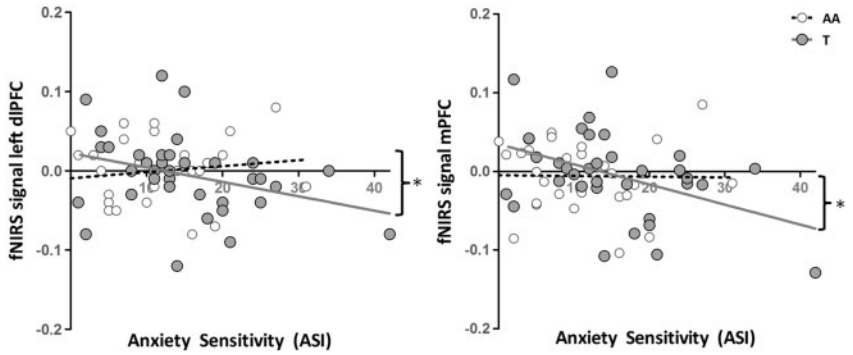

Fig. 3. Scatterplots showing *NPSR1* genotype (AA *vs* T) dependent significant correlations of left dlPFC activation (left) and mPFC activation (right) with anxiety sensitivity measured by the ASI in response to negative pictures. Asterisks indicate significant group differences for correlation coefficients ($z \geq 1.68$, $P < 0.05$).

aversive stimuli, the PFC involvement in response to positive emotions is rather under examined. Perlstein *et al.* (2002) proposed dlPFC activity in response to positive pictures to reflect activity of an appetitive system that is able to enhance cognitive functioning through an increased prefrontal dopamine turnover. In line with this notion, the tendency to over interpret the harmfulness of aversive events in T risk allele carriers (Raczka *et al.*, 2010) might also hinder positive pictures from being processed. Together with our finding of decreased prefrontal activity to positive pictures, future studies investigating *NPSR1* might also focus on deviant perception of positive emotions.

Interestingly, when additionally considering measures of anxiety (AS, state anxiety), AS and state anxiety were negatively correlated with mPFC and left dlPFC activation in response to negative pictures in *NPSR1* T allele carriers. Notably, both genotype groups in this study did not differ on anxiety measures *per se* (i.e. ASI and STAI, see Table 1) most probably reflecting the fact that healthy volunteers who were free of a diagnosis with anxiety disorders were investigated. However, in T risk allele carriers, the correlation between ASI and prefrontal activation was evident, while in AA homozygotes the correlation coefficient was close to zero (see Figure 3). Increased subclinical

anxiety thus was interpreted to lead to a decompensation of the previously adaptive compensatory upregulation of mPFC/dlPFC activity in healthy *NPSR1* T risk allele carriers. This is of interest since AS has been shown to be highly predictive of anxiety disorders especially panic disorder (Schmidt *et al*. 1997, 1999, 2006). The proposed maladaptive mPFC/dlPFC hypoactivity potentially reflecting an insufficient cortical top-down modulation during emotional processing (cf. Bishop *et al*., 2004; Bishop, 2009) against the background of a combined genetic and clinical-risk factor constellation is in line with previous reports of the *NPSR1* T allele being associated with increased AS in healthy probands with increased early adversity (Klauke *et al*., 2012) and panic disorder patients (Domschke *et al*., 2011). Furthermore, this interpretation is supported by the observation of decreased dlPFC activation in response to negative emotional stimuli in patients with clinically manifest panic disorder carrying the *NPSR1* T risk allele (Domschke *et al*., 2011). It thus can be speculated that the *NPSR1* T allele does not constitute a risk factor for pathological anxiety *per se*, since in healthy probands the assumed higher amygdala activity is suggested to be compensated by an upregulation of the PFC. This is not surprising considering the high prevalence of the *NPSR1* T allele. However, when *NPSR1* T allele carriers additionally exhibit high AS, this cognitive vulnerability to anxiety is suggested to impair the top-down modulation of the PFC entailing an increased risk of panic disorder. Thus, subclinical anxiety might impair the upregulation of the PFC to compensate for a subcortical fear response in T risk allele carriers potentially constituting a vulnerability factor for the development of panic disorder.

The following limitations have to be taken into account: The above interpretations of the results of our fNIRS study in an emotional n-back task are only justified in conjunction with functional MRI studies in complimentary emotional tasks. Imaging studies need to prove the speculated compensatory engagement of the PFC in the T group by demonstrating an increased functional coupling with the amygdala in the context of downregulating negative stimuli. Beyond, trial-by-trial valence reports should be included in such follow-up studies to further define the emotion regulation processes. Future investigations in larger, independent samples are warranted to replicate the suggested combined risk factor constellation. In particular also, this study was underpowered to investigate the described interaction between *NPSR1* and gender as an additional between-subject factor (cf. Domschke *et al*., 2011). The present sample consisted of university students and graduates. This might have been the reason why genotype groups did not show differences on the performance level although performance deficits can be expected when prefrontal compensation has reached its limit (cf. Siegmund *et al*., 2011).

In conclusion, this multi-level investigation of prefrontal cortex activity during emotional working memory and the interaction with premorbid anxiety supports a strong role of NPS and its receptor in the genetic and neural underpinnings of anxiety and anxiety disorders. In conjunction with comparable findings they may stimulate future studies exploring the potential of therapeutic agents targeting the NPS system in anxiety disorders (cf. Ionescu *et al*., 2012; Lukas and Neumann, 2012).

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