NeuroImage 71 (2013) 1–9

Contents lists available at SciVerse ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

Neuropeptide S receptor (*NPSR1*) gene variation modulates response inhibition and error monitoring

Christian Beste [a], Carsten Konrad [b], Christina Uhlmann [c], Volker Arolt [c], Peter Zwanzger [c], Katharina Domschke [d,*]

[a] Institute for Cognitive Neuroscience, Biopsychology, Ruhr-University of Bochum, D-44780 Bochum, Germany
[b] Department of Psychiatry and Psychotherapy, University of Marburg, Rudolf-Bultmann-Strasse 8, D-35039 Marburg, Germany
[c] Department of Psychiatry and Psychotherapy, University of Muenster, Albert-Schweitzer-Campus 1, Gebaeude A9, D-48149 Muenster, Germany
[d] Department of Psychiatry, Psychosomatics and Psychotherapy, University of Wuerzburg, Fuechsleinstrasse 15, D-97080 Wuerzburg, Germany

ARTICLE INFO

Article history:
Accepted 5 January 2013
Available online 11 January 2013

Keywords:
Neuropeptide S receptor
Genetics
Anxiety sensitivity
Executive functions
Event-related potentials (ERPs)

ABSTRACT

The neuropeptide S (NPS) system has been suggested to contribute to the pathogenesis of anxiety. In order to further characterize the cognitive-neurophysiological relevance of neuropeptide S in the etiology of anxiety, the influence of a functional neuropeptide S receptor gene (*NPSR1*) variant on response inhibition and error monitoring was investigated under consideration of the dimensional phenotype of anxiety sensitivity (AS).
In a sample of N = 97 healthy probands, event-related potential (ERP) measurement using a modified Flanker task was applied allowing for a distinct neurophysiological examination of processes related to response inhibition (Nogo-N2, Nogo-P3) and error monitoring (Ne/ERN). All subjects were genotyped for the functional *NPSR1* A/T ($Asn^{107}Ile$) variant (rs324981) and characterized for anxiety sensitivity using the Anxiety Sensitivity Index (ASI).
Carriers of the *NPSR1* T allele displayed intensified response inhibition (Nogo-P3) and error monitoring (Ne/ERN), which was in both cases paralleled by the behavioral data. Furthermore, anxiety sensitivity was found to be higher in *NPSR1* T allele carriers and to correlate with Nogo-P3 and Ne/ERN. A mediation analysis revealed the ERN to mediate the effect between *NPSR1* genotype and anxiety sensitivity.
In summary, the more active *NPSR1* T allele may confer enhanced response inhibition and increased error monitoring and might drive particularly error monitoring as a neurophysiological endophenotype of anxiety as reflected by increased anxiety sensitivity. These findings further corroborate a major role of the neuropeptide S system in the pathogenesis of anxiety and suggest a potentially beneficial use of therapeutic agents targeting the NPS system in anxiety disorders.

Introduction

Converging evidence from animal studies as well as molecular genetic, imaging genetic, gene–environment-interaction and multi-level systems studies in humans implicates the neuropeptide S (NPS) system as a novel pathomechanism in the etiology of anxiety (Reinscheid and Xu, 2005; for review see Okamura and Reinscheid, 2007; Pape et al., 2010).

In rodent models, centrally administered NPS has been observed to elicit increased arousal paralleled by an anxiolytic effect in anxiety-behavior-related tests like the open field, elevated plus maze, four-plate, elevated zero maze, light–dark box, recovery of righting reflex, stress-induced hyperthermia or defensive burying tests (e.g., Rizzi et al., 2008; Wegener et al., in press; Xu et al., 2004), with reciprocal findings in neuropeptide S receptor (NPSR) knock-out mice (Duangdao et al., 2009). In rats, NPS is primarily expressed in the locus coeruleus area, while NPSR has been reported to be widely expressed in various brain regions such as the amygdaloid complex, parts of the cingulate cortex and the ventral/lateral orbital cortex (Xu et al., 2007). NPS increases glutamatergic transmission to intercalated GABAergic neurons in the amygdala (Jüngling et al., 2008) and modulates the release of serotonin and norepinephrine in the frontal cortex (Raiteri et al., 2009) as well as extracellular levels of dopamine in the medial PFC and the nucleus accumbens (Mochizuki et al., 2010; Si et al., 2010), whereby NPS might influence anxiety- and arousal-related behavior.

In humans, the more active T allele of the functional neuropeptide S receptor (*NPSR1*) A/T Asn to Ile ($N^{107}I$) single nucleotide polymorphism (rs324981) (Bernier et al., 2006; Reinscheid et al., 2005) has consistently been reported to be associated with panic disorder (Domschke et al., 2011; Donner et al., 2010; Okamura et al., 2007). The *NPSR1* T risk allele was furthermore found to be related to increased heart rate as well as higher symptom reports and elevated anxiety sensitivity during a

* Corresponding author at: Department of Psychiatry, University of Wuerzburg, Fuechsleinstrasse 15, D-97080 Wuerzburg, Germany. Fax: +49 931 20177109.
E-mail address: Domschke_K@klinik.uni-wuerzburg.de (K. Domschke).

1053-8119/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neuroimage.2013.01.004

behavioral avoidance test as peripheral neurophysiological and dimensional endophenotypes of anxiety, respectively (Domschke et al., 2011). This increased anxiety sensitivity was suggested to be interactively influenced by the *NPSR1* A/T polymorphism and childhood maltreatment, with carriers of the more active *NPSR1* T/T genotype and a high number of maltreatment experiences in childhood reporting increased anxiety sensitivity (Klauke et al., in press). At first sight, the association of the gain-of-function *NPSR1* rs324981 T allele with panic disorder and anxiety sensitivity in humans seems inconsistent with findings in rodent models, where NPS and agonists at NPSR have been shown to exert a dose-dependent anxiolytic effect. However, in rodents NPS at the same time conferred increased arousal, which behaviorally and therefore also biochemically/genetically might be more comparable to human "anxiety" as in panic disorder or generalized anxiety disorder as compared the more phobia-related phasic "fear" (cf. Davis et al., 2010).

To further characterize the role of genetic factors in the pathogenesis of complex phenotypes in humans such as the role of *NPSR1* gene variation in anxiety, the investigation of endophenotypes on an intermediate level between genotype and clinical phenotype has proven to be useful (Gottesman and Gould, 2003). Event-related potentials (ERPs) comprising response inhibition (Nogo-N2/Nogo-P3) and error monitoring (Ne, "error negativity"/ERN, "error-related negativity") (Falkenstein et al., 1991; Gehring et al., 1993) are established markers of neurophysiological mechanisms underlying cognitive processes related to the selection and adaptation of behavior (Bush et al., 2000; Ridderinkhof et al., 2004b). Response inhibition (Nogo-P3) has been shown to be significantly associated with anxiety sensitivity (Sehlmeyer et al., 2010) and is hypothesized to be related to the behavioral inhibition system (BIS) (cf. Gray, 1982), which has been postulated as a potentially genetically driven neurophysiological risk factor of anxiety (e.g., Johnson et al., 2003; McDermott et al., 2009; Smoller and Tsuang, 1998; Smoller et al., 2001). Error monitoring processes (Ne/ERN) have consistently been suggested to constitute an endophenotype of anxiety-related psychopathology (Olvet and Hajcak, 2008) supported by several recent studies revealing error processing functions to be altered in e.g. trait anxiety and generalized anxiety disorder (Aarts and Pourtois, 2010; Weinberg et al., 2010, 2012; Xiao et al., 2011). Aside from the Ne/ERN, the second (P3-like) error positivity (Pe; Beste et al., 2008; Overbeeck et al., 2005) has been related to post-error adaptation (e.g., Nieuwenhuis et al., 2001) and shown to be diminished in anxious subjects (Moser et al., 2012).

Response inhibition processes are mediated by the orbitofrontal and dmPFC (Falkenstein, 2006; Ridderinkhof et al., 2004a); yet the relevance of the orbitofrontal and dmPFC varies across response inhibition subprocesses: the Nogo-N2 is supposed to mirror pre-motor inhibition or conflict monitoring processes (e.g., Beste et al., 2010c; Falkenstein, 2006; Nieuwenhuis et al., 2003) and has been shown to be generated in orbitofrontal areas (see: Falkenstein, 2006); the Nogo-P3 has been suggested to reflect the evaluation of a successful inhibition (Roche et al., 2005; Schmajuk et al., 2006) and has been shown to be additionally mediated via the ACC (e.g., Fallgatter et al., 2004), modulated by the meso-corticolimbic dopamine system (Beste et al., 2010b). Modulations in the Ne/ERN depend on anterior cingulate areas (review: Ridderinkhof et al., 2004a) and have been shown to alter the degree of post-error slowing (Debener et al., 2005) as an indicator of behavioral adaptation (Rabbitt, 1966). Yet, some studies failed to find a relation between Ne/ERN and post-error slowing (e.g., Gehring and Fencsik, 2001) with post-error slowing not always being adaptive (Notebaert et al., 2009). Thus, response inhibition/behavioral inhibition and error monitoring have been related to a neural network greatly overlapping with the brain fear circuit involving e.g. the ACC, the medial prefrontal cortex, the orbitofrontal cortex as well as the amygdala (see Bush et al., 2000; Charney and Deutch, 1996; Dresler et al., 2013; Fallgatter et al., 2004; Gorman et al., 1989, 2000; Huster et al., 2010; Pourtois et al., 2010; Ridderinkhof et al., 2004a). *NPSR1* mRNA is widely expressed in these brain regions such as the cingulate cortex, the orbitofrontal cortex and to a lesser degree also the amygdala (Xu et al., 2007; Allen Brain Atlas Resources [http://www.brain-map.org/], Seattle (WA): Allen Institute for Brain Science. ©2009) and therefore might directly or indirectly shape neural activity within this network. As furthermore *NPSR1* gene variation has been demonstrated to differentially impact activity of the amygdala, the dorsolateral prefrontal, lateral orbitofrontal and anterior cingulate cortex (ACC) during processing of anxiety-relevant emotional stimuli in healthy probands and patients with panic disorder, respectively (Dannlowski et al., 2011; Domschke et al., 2011), neural mechanisms underlying response inhibition and error monitoring might involve genetically driven networks of both executive cognitive and affective processes related to anxiety.

Given the suggested but mechanistically still not comprehensively understood role of the neuropeptide S system in anxiety-related phenotypes, in the present study we set out to further define the cognitive-neurophysiological role of neuropeptide S for the first time by investigating the influence of the functional *NPSR1* A/T (rs324981) variant on response inhibition (Nogo-N2; Nogo-P3) and error monitoring (Ne/ERN; Pe) as potential endophenotypes of anxiety as measured by anxiety sensitivity and state/trait anxiety. It was hypothesized that the more active *NPSR1* T risk allele would magnify response inhibition and error monitoring mediating an increased level of anxiety.

Material and methods

Subjects

A sample of N = 97 genetically unrelated subjects of Caucasian descent (f = 67; m = 30) was recruited for the study (cf. Beste et al., 2010a). The mean age of subjects was 25.16 ± 4.5 years. Absence of a history of neurological or psychiatric illness was assessed during an initial standardized screening interview by telephone. Second, absence of neurological and psychiatric disorders was established more thoroughly using a standardized life interview constructed for this study at the first personal visit. Our screening procedure excluded patients with any psychiatric disorders including substance abuse and anxiety disorders.

Anxiety sensitivity was measured by means of the German version of the Anxiety Sensitivity Index (ASI) (Alpers and Pauli, 2002; Reiss et al., 1986). Furthermore, anxiety was measured by the State-Trait Anxiety Inventory (STAI) (Laux et al., 1981), which consists of 40 statements differentiating between trait anxiety (TA) and the temporary condition of state anxiety (SA).

A blood sample (20 ml EDTA blood) was taken for genetic analyses. The study was approved by decision of the ethics committee of the University of Münster. All subjects gave written informed consent before any of the study procedures were commenced.

Genotyping

All subjects were genotyped for the neuropeptide S receptor gene (*NPSR1*) A/T ($N^{107}I$) variant (rs324981) according to published protocols (Domschke et al., 2011). Genotypes were determined by investigators blinded for phenotypes and independently by two investigators. Hardy–Weinberg criteria, assessed with the online available program DeFinetti (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl;Wienker TF and Strom TM; accessed April 2012), were fulfilled for *NPSR1* A/T genotype distribution in the present sample (AA: N = 29, AT: N = 50, TT: N = 18; $p > .6$).

Task

To examine a broad spectrum of response monitoring functions (i.e., response inhibition and error processing) we used a modified Flanker task. In this task the stimuli (arrowheads) were vertically arranged above and underneath a centrally presented fixation cross.

The target stimulus (arrowhead or circle) was presented in the center of the array with the arrowhead pointing to the left or right. The central stimulus was presented at the location of the fixation cross. The central stimulus was flanked by two vertically adjacent arrowheads which pointed in the same (compatible) or opposite (incompatible) direction as the target. Whenever an arrowhead was presented in the central position, the subjects had to press a response button with their left or right index finger depending on the direction of centrally presented arrowhead. Whenever a circle was presented at the central position, the subjects were required to refrain from responding (i.e., Nogo trials). The flankers preceded the target by 100 ms to maximize premature responding to the flankers to increase error likelihood. The target stimuli (arrowheads or circles) were displayed for 300 ms. The mean response–stimulus interval was 1600 ms and jittered between 1400 and 1800 ms. Flankers and target were switched off simultaneously. Time pressure was administered by asking the subjects to respond within 600 ms in order to further increase demands on conflict processing and response selection as well as to further increase response error likelihood. In trials with reaction times exceeding this deadline, a feedback stimulus (1000 Hz, 60 dB SPL) was given 1200 ms after the response; this stimulus had to be avoided by the subjects. Four blocks of 105 stimuli each were presented in this task. Compatible (60%) and incompatible stimuli (20%) and Nogo stimuli (circle) (20%) were presented randomly.

EEG recording and analysis

During the task the EEG was recorded from 24 Ag–AgCl electrodes according to the 10–20 System against a reference electrode located at Cz at a sampling rate of 500 Hz applying a filter bandwidth DC-80 Hz to the EEG. Electrode impedances were kept below 5 kΩ. Filtering was applied in the band-pass from 0.5 to 16 Hz (48 db/oct). In the epoched data, artifact rejection procedures were applied automatically with an amplitude threshold of ±80 μV. Horizontal and vertical eye movements were corrected using the Gratton–Coles Algorithm (Gratton et al., 1983) applied to the epoched data. Data was re-referenced to linked mastoids before a baseline correction was conducted (−200 ms till stimulus or response) and the average calculated. The EEG data was analyzed stimulus-locked and response-locked.

In the stimulus-locked data, the Nogo-N2 and Nogo-P3 were quantified. The Nogo-N2 was measured at electrodes Fz and FCz and quantified relative to pre-stimulus baseline. The Nogo-P3 was measured at electrodes FCz and Pz and also quantified against the pre-stimulus baseline. The Nogo-N2 was defined as the most negative peak between 200 and 350 ms post stimulus; the Nogo-P3 was defined as the most positive peak between 300 and 600 ms. Both components were quantified in amplitude and latency.

The Ne/ERN usually peaks around 50 until 110 ms post-response (Falkenstein et al., 1991). Within this interval we calculated the mean amplitude of the Ne/ERN as well as peak latency at electrodes Fz and FCz using a pre-response baseline −200 until 0 (i.e., time point of response). The Nc (i.e. post-response negativity occurring on correct trials) was quantified similarly. Ne/ERN and Nc were quantified across compatible and incompatible conditions. The Nogo condition was not used for the Ne analysis. The Pe was quantified at electrode Pz (e.g. Overbeeck et al., 2005). The Pe peaks between 200 and 500 ms post response. We calculated the mean amplitude of the Pe within this interval using a pre-response baseline −400 until −200. This baseline was chosen because of the P3b-like component around button press, which would have distorted a baseline from −200 until 0 (i.e. button press).

Statistics

Data was analyzed using (repeated measures) analyses of variance (ANOVAs). ERPs denoting conflict processing or response selection functions were analyzed using the within-subject factors "electrode" and depending on the potential "correctness" (error processing), "Go/Nogo" (response inhibition) and "compatibility" (conflict processing) as additional within-subject factors. *NPSR1* A/T "genotype" served as between-subject factor. Since previous results suggest that anxiety sensitivity modulates response inhibition processes (Sehlmeyer et al., 2010), but also varies across *NPSR1* A/T genotype groups (Domschke et al., 2011), regression analyses were calculated to estimate the relative influence of anxiety sensitivity and *NPSR1* A/T genotype on response inhibition and error monitoring processes. All variables subjected to analyses of variance were normally distributed as indicated by Kolmogorov–Smirnov Tests (all z's<.7; $p>.3$). In all analyses, Greenhouse–Geisser corrections were applied when appropriate. Post-hoc tests were Bonferroni-corrected, when necessary. For all analyses the means and standard error of mean (±SEM) are provided.

Results

Genetic and behavioral data

NPSR1 genotype groups did not differ in age ($p>.5$). A Kruskal–Wallis H test revealed that the distribution of sexes did not differ between genotype groups ($H=2.95$; $df=2$; $p>.2$). *NPSR1* genotype groups differed, however, regarding anxiety sensitivity ($F(2,94)=5.12$; $p=.017$; $\eta^2=.08$): AA genotype carriers displayed a significantly lower ASI score (14.15±2.2) than carriers of the AT (21.96±1.6) and TT (21.87±2.8) genotypes ($p<.02$), with the latter two genotype groups not significantly differing from each other ($p>.9$).

Reaction times (RTs in ms) were longer for correct (403±7) than for error trials (296±8) ($F(1,94)=893.95$; $p<.001$; $\eta^2=.90$) and also longer for correct incompatible than for correct compatible trials ($F(1,94)=170.63$; $p<.001$; $\eta^2=.64$). In both cases, *NPSR1* genotype did not modulate the pattern of results, as revealed by non-significant interactions (all $F<1.15$; $p>.3$; $\eta^2<.03$). Genotype groups did not differ in their overall RT on correct trials ($F(2,94)<0.85$; $p>.5$; $\eta^2=.03$) and on error trials ($F(2,94)<0.5$; $p>.6$; $\eta^2=.03$). To examine the degree of behavioral adaptation after the commitment of a response error, 'post-error slowing' was calculated (Rabbitt, 1966). Therefore, we subjected the mean reaction time of correct responses in succession and those after an error ("sequence") to a mixed measures ANOVA with "group" as between-subject factor. RTs on correct response after an error were significantly longer (400±7) than RTs on correct responses in succession (367±7) ($F(1,94)=596.2$; $p<.001$; $\eta^2=.86$). This slowing effect was different across *NPSR1* genotype groups, as indicated by a significant interaction ($F(2,94)=13.01$; $p<.001$; $\eta^2=.21$). Submitting the difference in RTs between correct responses in succession and those after an error to a univariate ANOVA revealed that each genotype group differed from each other in an allele-dose fashion ($F(2,94)=11.61$; $p<.001$; $\eta^2=.19$). Bonferroni-corrected pair-wise comparisons showed that the AA genotype conferred the lowest post-error slowing effect (23.6±2), followed by the AT (31.9±2) and TT (41±3) genotype groups ($p<.018$) (Fig. 1A). Correlational analyses revealed that the degree of post-error slowing was stronger, when the ASI score was higher ($r=.46$; $R^2=.21$; $p<.001$). Using the STAI, no significant correlations were evident for the 'trait score' and 'state score' ($r<.1$; $p>.5$).

We further analyzed, whether the amount of errors committed occurred mainly after correct trials or after error trials (i.e., post-error accuracy). The analysis on post-error accuracy showed that 30.51% (±10) of errors occurring after the previous trials also showed an error, while 69.49% (±10) of errors occurring after the previous trial were correct ($p<.001$). Moreover, there was a negative correlation between post-error slowing and post-error accuracy. In particular, this negative correlation showed that the longer the mean post-error slowing, the lower the frequency of errors was (i.e., the higher the post-error accuracy) ($r=-.45$; $R^2=.20$; $p=.001$). Error rates (percent errors) were lower in the compatible (3.1±0.3) than in the incompatible (8.7±0.4) condition ($F(2,94)=104.93$; $p<.001$; $\eta^2=.52$). This effect was not differentially modulated across genotype groups, as indicated by

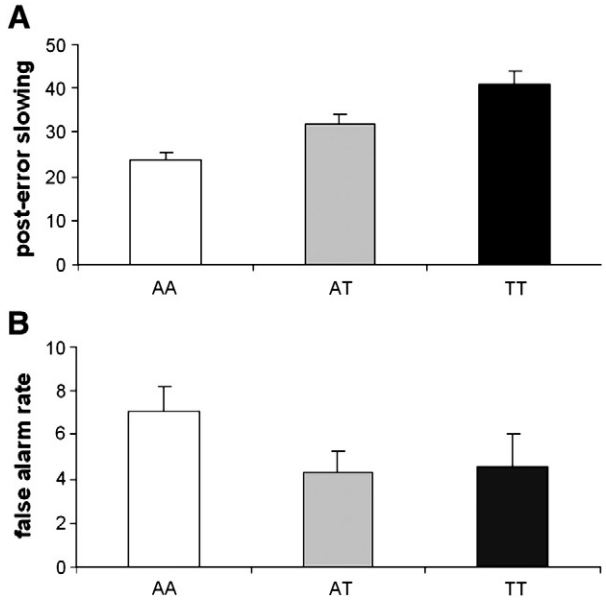

Fig. 1. Variation in (A) the degree of post-error slowing and (B) the rate of false alarms across *NPSR1* rs324981 A/T genotype groups.

the non-significant interaction ($F(2,94)=2.12$; $p>.12$; $\eta^2=.04$). Regarding the post-error accuracy, there were genotype group effects ($F(2,94)=6.61$; $p<.001$; $\eta^2=.12$) showing that the post-error accuracy (based on the total amount of errors) was higher (i.e., errors were lower) in the TT genotype group (8.5 ± 0.4), compared to the AT (15.6 ± 0.6) and AA genotype groups (14.8 ± 0.5) ($p<.001$). The latter two did not differ from each other ($p>.3$). This pattern is in line with the correlational analysis on post-error slowing and post-error accuracy. Further correlation analyses revealed that the degree of post-error accuracy was stronger, when the ASI score was higher ($r=.33$; $R^2=.10$; $p<.001$). Using the STAI, no significant correlations were evident for the 'trait score' and 'state score' ($r<0.9$; $p>.5$).

For the frequency of false alarms, ANOVA revealed differences across *NPSR1* genotypes ($F(2,94)=4.88$; $p=.01$; $\eta^2=.09$). Bonferroni-corrected pair-wise comparisons showed that the AA genotype group committed more false alarms (7.1 ± 0.7) ($p=.009$) than the AT (4.3 ± 0.5) or TT (4.6 ± 0.9) genotype group, which did not differ from each other (Fig. 1B). There was generally no relation between the degree of post-error slowing and the rate of false alarms ($r=.13$; $p>.4$). Further correlational analyses revealed that the rate of false alarms was lower in subjects with a higher ASI score ($r=.39$; $R^2=0.15$; $p<.001$), replicating previous results (Sehlmeyer et al., 2010). No correlations were evident with the STAI 'trait score' and 'state score' (all $r<.1$; $p>.5$).

Neurophysiological data

Stimulus-locked analysis

For the N2-ERP, the mixed effects ANOVA revealed that this potential was larger (i.e., more negative) at electrode Fz (-1.64 ± 0.1), compared to FCz (-0.49 ± 0.2) ($F(1,94)=83.43$; $p<.001$; $\eta^2=.47$). N2 potentials were also more negative for Nogo (-3.24 ± 0.2) compared to Go (1.1 ± 0.2) trials ($F(1,94)=311.05$; $p<.001$; $\eta^2=.76$). However, the Go/Nogo effect was not further modulated by *NPSR1* genotype ($F(2,94)=1.89$; $p=.116$; $\eta^2=.04$). There was also no main effect of genotype group ($F(1,94)=1.05$; $p>.2$). For the Nogo-N2, there was no correlation with the ASI score ($r<.2$; $p>.4$).

For the P3-ERP, a similar ANOVA revealed a main effect "Go/Nogo" ($F(1,94)=8.80$; $p=.004$; $\eta^2=.08$) showing that the potential was overall larger on Nogo (12.26 ± 0.4) compared to Go trials (11.49 ± 0.3). Yet, an interaction "Go/Nogo × electrode" revealed that this effect was different for electrode FCz and Pz ($F(1,94)=101.53$; $p<.001$; $\eta^2=.51$). Bonferroni-corrected post-hoc tests (paired samples t-tests) showed that for electrode FCz the Nogo potential (14.65 ± 0.5) was stronger than the Go potential (9.1 ± 0.3) ($t=10.47$; $df=96$; $p<.001$), while for electrode Pz the Go potential (13.92 ± 0.4) was larger than the Nogo potential (9.96 ± 0.5) ($t=-7.98$; $df=96$; $p<.001$).

Importantly, there was an interaction "Go/Nogo × genotype" ($F(2,94)=4.49$; $p=.014$; $\eta^2=.09$) (see Fig. 2A). Subsequent univariate ANOVAs revealed that the Nogo-P3 differed across *NPSR1* genotype groups ($F(2,94)=11.33$; $p<.001$; $\eta^2=.19$), but there were no genotype group differences on Go trials ($F(2,94)=1.51$; $p>.2$; $\eta^2=.03$). The Nogo-P3 was lower in the AA (11.10 ± 0.4) compared to AT (13.33 ± 0.3) and TT (13.92 ± 0.5) genotype groups. Both, the AT and TT genotype groups differed from the AA genotype group ($p<.001$), but not from each other ($p>.9$).

A first correlation analysis using the Nogo-P3 amplitude as predictor for the ASI score revealed a substantial positive correlation ($r=.461$; $R^2=.21$; $p<.001$) showing that a higher ASI score was related to a stronger Nogo-P3 (cf. Sehlmeyer et al., 2010) (see Fig. 2B). The correlation remained similar, when corrected for outliers ($r=.441$; $R^2=.19$; $p<.001$). Furthermore, a step-wise regression analysis ('forward method'), in which ASI and genotype group were stepwise entered into the model, was calculated. The regression analysis is useful, since both factors (ASI and genotype group) were not independent from each other

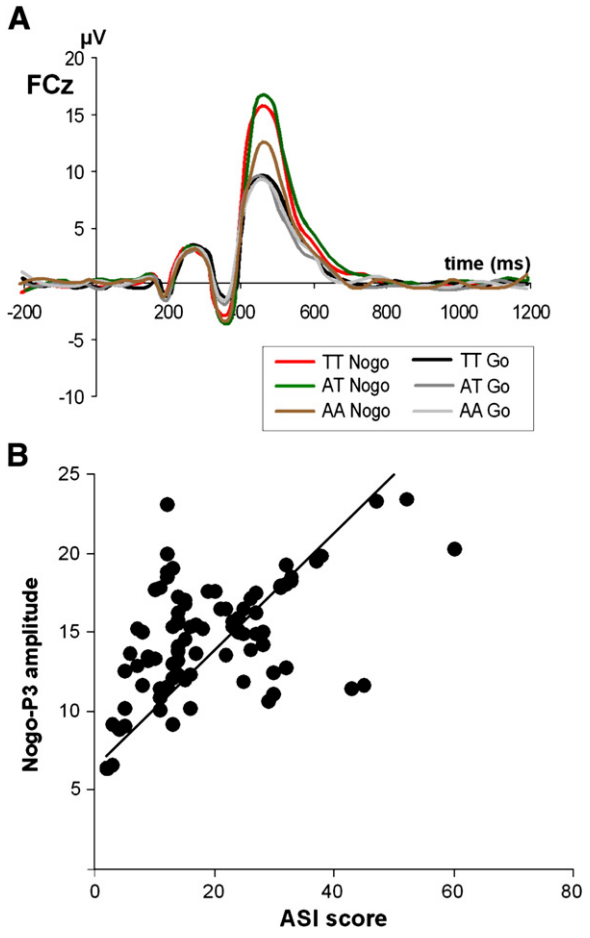

Fig. 2. (A) Stimulus-locked ERPs at electrode FCz. Time point 0 denotes the time point of response execution. The different ERP traces denote the potential on Go and Nogo trials for each *NPSR1* rs324981 A/T genotype group. (B) Scatterplot showing the correlation of the Nogo-P3 amplitude and anxiety sensitivity (ASI) score.

with increased ASI scores in *NPSR1* T allele carriers (see above). The step-wise regression revealed that the model fit was better ($\Delta R^2 = 4.7\%$) when both factors (ASI and genotype group) were entered into the model ($R^2 = 0.259$; $F(2,94) = 15.56$; $p < .001$) as compared to when only ASI was used as predictor ($R^2 = 0.212$). However, the relative influence of the ASI score was nearly twice as large ($\beta = .404$) as the influence of genotype group ($\beta = .224$). There was no correlation between the STAI trait/state score and the Nogo-N2 or Nogo-P3 amplitudes (all $r < .2$; $p > .3$).

Response-locked analysis: Ne/ERN and Pe

Post-response ERP amplitudes were larger for error (-7.1 ± 0.2), than for correct trials (-4.7 ± 0.2) ($F(1,94) = 77.72$; $p < .001$; $\eta^2 = .45$). ERPs were also larger at electrode FCz (-6.22 ± 0.2), compared to Fz (-5.56 ± 0.2) ($F(1,94) = 8.21$; $p = .005$; $\eta^2 = .08$). There was a main effect of "genotype" ($F(2,94) = 7.97$; $p = .001$; $\eta^2 = .14$) showing that post-response ERPs were largest for the TT genotype group (TT: -6.65 ± 0.3; AT: -5.88 ± 0.1; AA: -5.14 ± 0.2). Bonferroni-corrected pair-wise comparison revealed that the TT genotype differed from the other genotype groups ($p < .043$), while AT and AA genotype groups did not differ from each other ($p > .1$).

Importantly, there was an interaction "correctness × genotype" at electrode FCz ($F(2,94) = 7.73$; $p = .001$; $\eta^2 = .14$) (see Fig. 3A). Subsequent univariate ANOVAs as post-hoc tests revealed that *NPSR1* genotype groups differed in their Ne/ERN amplitude ($F(1,94) = 9.72$; $p < .001$; $\eta^2 = .17$), but not on the Nc amplitude ($F(1,94) = 0.74$; $p > .7$; $\eta^2 = .02$). Bonferroni-corrected pair-wise comparisons for the Ne/ERN showed that the Ne/ERN war strongest in the TT genotype group (-8.49 ± 0.5), followed by the AT (-6.91 ± 0.3) and AA genotype groups (-5.6 ± 0.4) (all $p < .030$).[1] Since previous studies reported effects of gender on the Ne/ERN, this factor was evaluated separately. This analysis revealed no main effect of gender or an interaction with genotype on the Ne/ERN (all $F < 1$; $p > .4$; $\eta^2 < .03$). A correlation analysis revealed an inverse relationship between ASI score and Ne/ERN amplitude ($r = -.486$; $R^2 = .23$; $p < .001$), showing that higher ASI scores were related to higher (i.e., more negative) Ne/ERN amplitudes at electrode FCz (see Fig. 3B). As with the previous analysis on response inhibition data, the correlation remained similar after correction for outliers ($r = .466$; $R^2 = .21$; $p < .001$). The model fit of the step-wise regression model was better ($\Delta R^2 = 7.5\%$), when besides the ASI score, the genotype was fed into the model ($R^2 = 0.311$; $F(2,94) = 19.41$; $p < .001$). The influence of ASI was nearly twice as large ($\beta = .417$) as the influence of genotype group ($\beta = .238$). There were generally no significant correlations between the Pe and the ASI or the STAI trait and state score and between the Ne/ERN and the STAI trait and state score (all $r < .2$; $p > .4$).

Analyzing the potentials at electrode Pz to determine the Pe (see Fig. 4), no interaction "correctness × genotype" ($F(2,94) = 1.13$; $p > .2$; $\eta^2 = .02$) and no main effect of genotype group ($F(2,94) = 0.11$; $p > .8$; $\eta^2 = .01$) could be discerned. This shows that the Pe was not differentially modulated across *NPSR1* genotype groups.

Mediation analyses

Additionally, a mediation analysis (cf. Preacher and Hayes, 2004) was performed to test whether Nogo-P3 and Ne/ERN amplitudes at electrode FCz mediated the effect between genotype and anxiety sensitivity, respectively, by calculating the difference between the regression coefficients in a simple regression (genotype predicts ASI, c) and the coefficient when Nogo-P3 and ERN, respectively, were also fed into the model (c′) and testing it against zero (i.e., $c - c' = 0$) using the Sobel test (Sobel, 1982). Here, it could be shown that the ERN mediated the effect between *NPSR1* genotype group and ASI score ($z = 2.05$; $p = .04$). For the Nogo-P3, there were generally no mediation effects ($z < .85$; $p > .4$).

[1] As can be seen in the scatterplot of Fig. 3, there are some outliers in the distribution that may affect the results obtained. However, after removing these outliers, the effects of *NPSR1* genotype remained stable. However, it may also be argued that the AA genotype group revealed higher error rates, which may slightly skew the comparison with the TT group. We re-run this part of the analysis and took the first N error trials of the AA subjects matched to the TT subject error rates. When comparing the Ne/ERN amplitudes, the Ne/ERN was still lower (-6.3 ± 0.9) in the AA than in the TT genotype group (-7.44 ± 0.6) ($p < .01$).

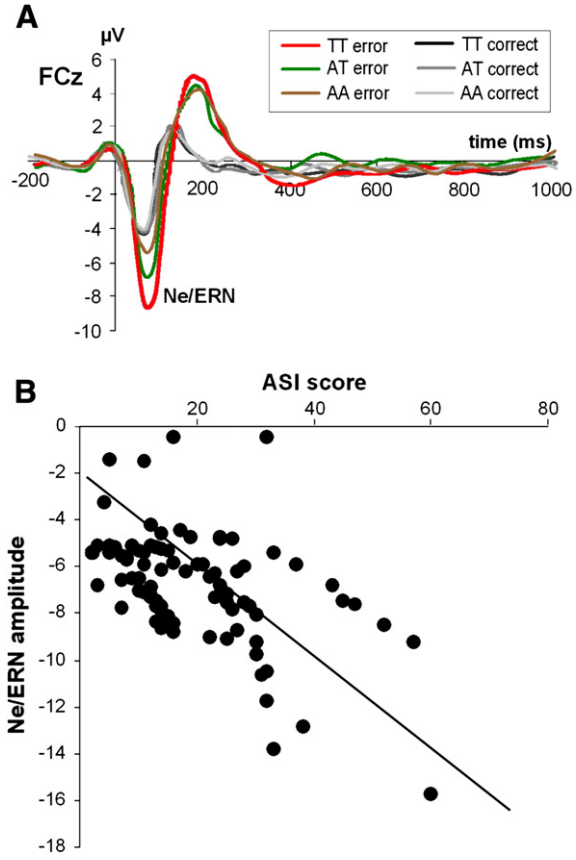

Fig. 3. (A) Response-locked ERPs at electrode FCz. Time point 0 denotes the time point of response execution. The different ERP traces denote the potential on correct (Nc) and error trials (Ne/ERN) for each *NPSR1* rs324981 A/T genotype group. (B) Scatterplot showing the correlation of the Ne/ERN amplitude and anxiety sensitivity (ASI) score.

Discussion

The present results show that response inhibition and error monitoring processes are differentially affected by the functional *NPSR1* rs324981 A/T genotype. In particular, it could be demonstrated that neurophysiological processes related to response inhibition (Nogo-P3)

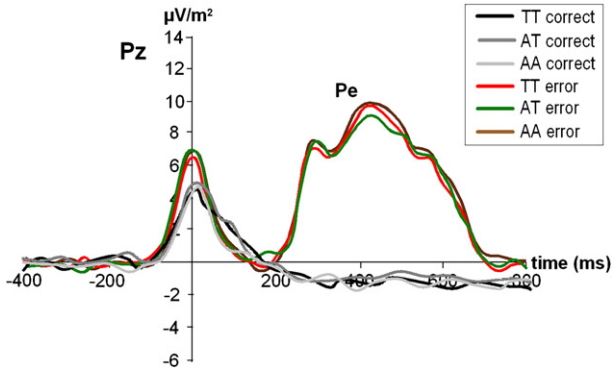

Fig. 4. Response-locked ERPs at electrode Pz. Time point 0 denotes the time point of response execution. The different ERP traces denote the potential on correct and error trials (Pe) for each *NPSR1* rs324981 A/T genotype group.

and error monitoring (Ne/ERN) are intensified in carriers of the more active *NPSR1* TT risk genotype, which parallels the behavioral data pattern, i.e. increased post-error slowing and increased post-error accuracy (i.e., fewer errors in trials following an error trial) in carriers of the more active *NPSR1* T allele, which was furthermore associated with increased anxiety sensitivity. Therefore, an adaptive behavior could be hypothesized to be elevated in non-pathological, but risk-increasing conditions (anxiety sensitivity), which in extreme cases (anxiety disorders) may become maladaptive. The low rate of false alarms on Nogo-trials in T allele carriers seems to indicate a dominant model for the T allele to render response inhibition more efficient than the AA genotype. Error monitoring processes were affected in an allele-dose fashion, with increasing behavioral adaptation depending on the number of T alleles. With regard to the dimensional phenotype of anxiety sensitivity (AS), *NPSR1* T allele carriers displayed a significantly higher AS score than AA homozygotes. Additionally, regression analyses revealed that anxiety sensitivity impacted Nogo-P3 and Ne/ERN beyond the influence of *NPSR1* genotype. Finally, a mediation analysis revealed the ERN to mediate the effect between *NPSR1* genotype and anxiety sensitivity. For the Nogo-P3, no mediation effect was detectable suggesting the ERN to be more of an important factor in this gene–anxiety association than the Nogo-P3.

The *NPSR1* T allele, being associated with increased anxiety sensitivity, and—beyond a genetic effect—anxiety sensitivity itself may confer enhanced response inhibition and thus an over-activation of the behavioral inhibition system (BIS) leading to enhanced behavioral inhibition, increased arousal and attention to outputs (cf. Gray, 1982), which are suggested as potentially genetically driven neurophysiological risk factors of anxiety (e.g., Johnson et al., 2003; McDermott et al., 2009; Smoller and Tsuang, 1998; Smoller et al., 2001). The present results are in line with several previous findings of the *NPSR1* T allele to be associated with anxiety sensitivity, arousal and sustained fear related phenotypes such as panic disorder (Domschke et al., 2011; Domschke et al., 2012b; Donner et al., 2010; Klauke et al., in press; Okamura et al., 2007). They are furthermore in accordance with reports of increased cognitive control and enhanced evaluation of their behavioral outcomes as reflected by increased Nogo-P3 responses and fewer false alarm rates, respectively, in probands with high anxiety sensitivity (Sehlmeyer et al., 2010). No effect of *NPSR1* genotype was detected on the Nogo-N2 ERP. As *NPSR1* gene variation seems to primarily drive anxiety sensitivity related states (Domschke et al., 2011) and the Nogo-N2 has previously been reported not to be influenced by anxiety sensitivity but rather by trait anxiety (Sehlmeyer et al., 2010), the present finding supports the hypothesis of differential effects of different anxiety constructs on Nogo-N2 and -P3 components. The increased Nogo-P3 response might thus mirror strong attentive and evaluative components regarding response inhibition as well as potentially anxiety-relevant physical and psychological processes driven by the *NPSR1* T risk allele (cf. Beste et al., 2011; Domschke et al., 2010; Roche et al., 2005). On a neurotransmitter level, central administration of neuropeptide S (NPS) has been observed to dose-dependently increase extracellular levels of dopamine in the medial prefrontal cortex and the shell of the nucleus accumbens (Mochizuki et al., 2010; Si et al., 2010). Comparing diseases exerting differential dysfunctions of the nigro-striatal and meso-corticolimbic dopamine system it has been shown that the Nogo-P3, but not the Nogo-N2 is modulated by the meso-corticolimbic dopamine system (Beste et al., 2010b). This suggests that these response inhibition sub-processes seem to be mediated via distinct neural circuits (Beste et al., 2010c, 2010d). An alternative explanation may be that larger Nogo-P3 amplitudes reflect intensified effort (i.e., less efficient performance) to inhibit the response. Roche et al. (2005), for example, found that absent-minded individuals exhibit larger Nogo-P3 amplitudes and suggested that these individuals may require greater effort to perform the task than individuals more focused on the task. Thus, *NPSR1* T allele carriers may have exhibited inefficient inhibition processes, evidenced by greater P3 amplitudes.

The Ne/ERN was presently found to be modulated in an allele-dose fashion, with stronger Ne/ERN and more efficient post-error behavioral adaptation along with an increasing number of *NPSR1* T alleles.[2] The Ne/ERN is generated in the ACC (Bush et al., 2000; Ridderinkhof et al., 2004a) on the basis of a phasic dopaminergic signal elicited in the basal ganglia (Holroyd and Coles, 2002) suggesting a strong influence of the dopaminergic system on the Ne/ERN (review: Jocham and Ullsperger, 2008). The Ne/ERN has consequently been reported to be decreased in diseases accompanied by dopamine dysfunction such as Parkinson's disease (e.g., Willemssen et al., 2008, 2009) or Huntington's disease (e.g., Beste et al., 2006, 2009), as well as in schizophrenia (Foti et al., 2012). As NPS has been shown to increase extracellular levels of dopamine in the medial PFC and the nucleus accumbens (see above; Mochizuki et al., 2010; Si et al., 2010), the more active *NPSR1* T allele might increase error monitoring (Ne/ERN) via elevated dopamine levels. The fact that—opposed to response inhibition processes—the Ne/ERN follows the pharmacologically proven dose-dependent increase of extracellular dopamine level in the medial PFC (Si et al., 2010) suggests that the neurophysiological mechanisms mediating error processing are more fine-tuned than mechanisms related to response inhibition potentially driven by *NPSR1* gene variation. NPS has also been shown to be strongly expressed in the locus coeruleus region and thus to be tightly linked to the central noradrenergic system (Raiteri et al., 2009; Xu et al., 2004). Recent pharmacological results provide evidence for the noradrenergic system to affect error monitoring (Graf et al., 2011; Jocham and Ullsperger, 2008). NPS may therefore affect error processing not only via modulation of the dopaminergic, but also of the noradrenergic system. The Ne/ERN has furthermore been found to mediate the association between *NPSR1* genotype group and anxiety sensitivity suggesting error monitoring as a potential endophenotype of anxiety sensitivity or anxiety sensitivity related phenotypes such as panic disorder (Schmidt et al., 2006) or generalized anxiety disorder (Rector et al., 2007), while no mediation effect was detectable for the Nogo-P3. As the noradrenergic system—tightly linked with neuropeptide S activity (Raiteri et al., 2009; Xu et al., 2004)—has been shown to influence error monitoring to a greater extent than response inhibition (Graf et al., 2011; Jocham and Ullsperger, 2009) and as neuropeptide S receptor gene variation has repeatedly shown to be associated with anxiety sensitivity (this study; Domschke et al., 2011), the gene–anxiety mediation effect by the ERN might be driven by noradrenergic mechanisms.

Interestingly, the Pe did not show *NPSR1* genotype dependent variations, despite the Pe has been suggested to be generated in the ACC (van Veen and Carter, 2002) and has been shown to be altered in anxious subjects (Moser et al., 2012). Apparently, the ERN and the Pe are not only dissociable with respect to the cognitive processes they reflect (Moser et al., 2011; Steinhauser and Yeung, 2010), but also due to their neurobiological substrates. This is in line with other data suggesting that the Pe is related to processes in the anterior insula (Ullsperger et al., 2010) and may reflect processes similar to processes captured by the P3 (Ridderinkhof et al., 2009).

The present study should be considered in the light of some limitations: For exclusion of mental disorders a standardized life interview constructed for this study was applied, but no validated interview such as the SCID or the MINI. Also, while anxiety sensitivity (AS) was found to correlate with *NPSR1* genotype as well as Nogo-P3 and Ne/ERN, no correlation with STAI scores could be discerned. Anxiety sensitivity and trait anxiety as captured by the STAI can be considered

[2] The issue what processes are reflected by post-error slowing has been debated (e.g., Notebaert et al., 2009). Moreover, there was a negative correlation between post-error slowing and post-error error rate. In particular, this negative correlation showed that the longer the mean post-error slowing, the lower the frequency of errors was. Also, errors mainly occurred after correct trials and not after a previous error trial. This suggests that post-error slowing reflects behavioural adaptation.

different constructs of anxiety-related measures with only low to modest overlapping variance (McNally, 1996). While AS reflects fear of particularly bodily anxiety symptoms and is suggested as a predictor of anxiety disorders, especially panic disorder (Schmidt et al., 2006) and worry-related generalized anxiety disorder (Rector et al., 2007), STAI-T has been shown to predict future anxiety in general based on previous anxiety experiences not specific to panic disorder (Reiss, 1997; Taylor et al., 1991; Taylor et al., 1992). Given evidence for ERN enhancement to be relatively specific to the anxious apprehension (i.e., worry), trait anxiety and generalized subtype of anxiety (Aarts and Pourtois, 2010; Hajcak et al., 2003; Moran et al., 2012; Moser et al., 2012; Vaidyanathan et al., 2012) and for the STAI to correlate with the ERN (e.g., Riesel et al., 2012; Vocat et al., 2008), the current results not showing association with the STAI are at first sight at odds with these studies. However, as anxiety sensitivity has also been tightly linked with worry and generalized anxiety disorder (Rector et al., 2007), the present study is not that divergent from previous studies. In fact, the present study might contribute to the more precise definition of neurophysiological endophenotypes underlying the presumably differential neurobiological pathways of "phasic" and "sustained" fear, related to the clinical entities of phobias on the one hand and panic disorder/generalized anxiety disorder, possibly linked to anxiety sensitivity, on the other hand (cf. Davis et al., 2010) and the potential role of *NPSR1* gene variation and ERN in particularly mediating "sustained" fear/panic disorder related phenotypes. Also, for a number of reasons previous studies showing association of ERN with STAI scores are not fully comparable to the present study, which may partly explain differences in results: The study by Vocat et al. (2008) used a Go/Nogo task, where errors were nearly equally frequent to correct trials. This effect was desired by the authors in order to have more error trials for the analysis. However, it is known that the ERN becomes more similar to the CRN, when errors are highly frequent (e.g., Band and Kok, 2000; Pailing and Segalowitz, 2004). This experiment therefore differs from our experiment, where there was no such high error rate (approx. 8%). The study by Riesel et al. (2012) also differed from our study in using a punishment manipulation. The aim of the Riesel et al. study was to examine, whether the ERN is enhanced in conditions in which errors are punished. Obviously, this manipulation put larger emphasis on anxious apprehension related processes as it was the case in our study. Moreover, correlations with the STAI score obtained in the Riesel et al. study were based on a difference measure, where the ERN in a control condition was subtracted from the ERN in the punishment condition. Also, while the mean ASI score in the present sample (19.76, SD: 11.97) corresponds to the norm mean ASI score (Peterson and Reiss, 1992), the mean STAI-trait score in the present sample was 34.35 (SD: 7.95), which is significantly lower than the norm mean STAI-trait scores (38.07 (SD: 8.20) in N = 332 males, 38.22 (SD: 8.20) in N = 644 females) given in the Manual for the State-Trait Anxiety Inventory (Spielberger et al., 1970) ($t = 3.96$ (males), $t = 4.35$ (females); both $p < 0.0001$). This might indicate super-normality of the present sample for the STAI-trait and therefore does not allow for full comparability of the results to other populations. Finally, as recent results show that the ERN is modulated by the individual degree of neuroticism (Olvet and Hajcak, 2012), systematically controlling for this potentially confounding factor in future studies might be worthwhile. With regard to limitations of the present study on a molecular level, the present results have not been controlled for polymorphisms of the adenosinergic, dopaminergic system and serotonergic system, which have previously been suggested to in part drive error monitoring and/or behavioral inhibition or to interact with the neuropeptide S system (Beste et al., 2011; Braet et al., 2011; Domschke et al., 2012a, 2012b; Filbey et al., 2012; Stokes et al., 2011), which could be subject to further studies.

In summary, the more active *NPSR1* T allele may confer enhanced response inhibition and increased error monitoring and might drive particularly error monitoring as a neurophysiological endophenotype of anxiety as reflected by increased anxiety sensitivity. In synopsis with previous animal studies as well as molecular genetic, imaging genetic, gene–environment-interaction and multi-level systems studies in humans, the present findings further corroborate a pathomechanistic role of the NPS system as well as a potentially beneficial use of therapeutic agents targeting the NPS system in anxiety disorders.

Acknowledgment

This research was supported by grants from the Deutsche Forschungsgemeinschaft (DFG) to C.B. (BE4045/10-1), K.D. (SFB-TRR-58, project C2) and P.Z. (SFB-TRR-58, project C1). We gratefully acknowledge the skilful technical support by Ms. Kathrin Schwarte.

References

Aarts, K., Pourtois, G., 2010. Anxiety not only increases, but also alters early error-monitoring functions. Cogn. Affect. Behav. Neurosci. 10, 479–492.
Alpers, G.W., Pauli, P., 2002. Angstsensitivitäts-Index. PsychScience, Würzburg.
Band, G.P., Kok, A., 2000. Age effects on response monitoring in a mental-rotation task. Biol. Psychol. 51, 201–221.
Bernier, V., Stocco, R., Bogusky, M.J., Joyce, J.G., Parachoniak, C., Grenier, K., Arget, M., Mathieu, M.C., O'Neill, G.P., Slipetz, D., Crackower, M.A., Tan, C.M., Therien, A.G., 2006. Structure-function relationships in the neuropeptide S receptor: molecular consequences of the asthma-associated mutation N107I. J. Biol. Chem. 281, 24704–24712.
Beste, C., Saft, C., Andrich, J., Gold, R., Falkenstein, M., 2006. Error processing in Huntington's disease. PLoS One e86.
Beste, C., Saft, C., Konrad, C., Andrich, J., Habbel, A., Schepers, I., Jansen, A., Pfleiderer, B., Falkenstein, M., 2008. Levels of error processing in Huntington's disease: a combined study using event-related potentials and voxel-based morphometry. Hum. Brain Mapp. 29, 121–130.
Beste, C., Willemssen, R., Saft, C., Falkenstein, M., 2009. Error processing in normal aging and in basal ganglia disorders. Neuroscience 159, 143–149.
Beste, C., Baune, B.T., Domschke, K., Falkenstein, M., Konrad, C., 2010a. Dissociable influences of NR2B-receptor related neural transmission on functions of distinct associative basal ganglia circuits. Neuroimage 52, 309–315.
Beste, C., Baune, B.T., Domschke, K., Falkenstein, M., Konrad, C., 2010b. Paradoxical association of the brain-derived-neurotrophic factor val66met genotype with response inhibition. Neuroscience 166, 178–184.
Beste, C., Domschke, K., Kolev, V., Yordanova, J., Baffa, A., Falkenstein, M., Konrad, C., 2010c. Functional 5-HT1a receptor polymorphism selectively modulates error-specific subprocesses of performance monitoring. Hum. Brain Mapp. 31, 621–630.
Beste, C., Willemssen, R., Saft, C., Falkenstein, M., 2010d. Response inhibition subprocesses and dopaminergic pathways: basal ganglia disease effects. Neuropsychologia 48, 366–373.
Beste, C., Domschke, K., Radenz, B., Falkenstein, M., Konrad, C., 2011. The functional 5-HT1A receptor polymorphism affects response inhibition processes in a context-dependent manner. Neuropsychologia 49, 2664–2672.
Braet, W., Johnson, K.A., Tobin, C.T., Acheson, R., McDonnell, C., Hawi, Z., Barry, E., Mulligan, A., Gill, M., Bellgrove, M.A., Robertson, I.H., Garavan, H., 2011. fMRI activation during response inhibition and error processing: the role of the DAT1 gene in typically developing adolescents and those diagnosed with ADHD. Neuropsychologia 49, 1641–1650.
Bush, G., Luu, P., Posner, M.I., 2000. Cognitive and emotional influences in anterior cingulate cortex. Trends Cogn. Sci. 4, 215–222.
Charney, D.S., Deutch, A., 1996. A functional neuroanatomy of anxiety and fear: implications for the pathophysiology and treatment of anxiety disorders. Crit. Rev. Neurobiol. 10, 419–446.
Dannlowski, U., Kugel, H., Franke, F., Stuhrmann, A., Hohoff, C., Zwanzger, P., Lenzen, T., Grotegerd, D., Suslow, T., Arolt, V., Heindel, W., Domschke, K., 2011. Neuropeptide-S (NPS) receptor genotype modulates basolateral amygdala responsiveness to aversive stimuli. Neuropsychopharmacology 36, 1879–1885.
Davis, M., Walker, D.L., Miles, L., Grillon, C., 2010. Phasic vs sustained fear in rats and humans: role of the extended amygdala in fear vs anxiety. Neuropsychopharmacology 35, 105–135.
Debener, S., Ullsperger, M., Siegel, M., Fiehler, K., von Cramon, D.Y., Engel, A.K., 2005. Trial-by-trial coupling of concurrent electroencephalogram and functional magnetic resonance imaging identifies the dynamics of performance monitoring. J. Neurosci. 25, 11730–11737.
Domschke, K., Stevens, S., Pfleiderer, B., Gerlach, A.L., 2010. Interoceptive sensitivity in anxiety and anxiety disorders: an overview and integration of neurobiological findings. Clin. Psychol. Rev. 30, 1–11.
Domschke, K., Reif, A., Weber, H., Richter, J., Hohoff, C., Ohrmann, P., Pedersen, A., Bauer, J., Suslow, T., Kugel, H., Heindel, W., Baumann, C., Klauke, B., Jacob, C., Maier, W., Fritze, J., Bandelow, B., Krakowitzky, P., Rothermundt, M., Erhardt, A., Binder, E.B., Holsboer, F., Gerlach, A.L., Kircher, T., Lang, T., Alpers, G.W., Ströhle, A., Fehm, L., Gloster, A.T., Wittchen, H.U., Arolt, V., Pauli, P., Hamm, A., Deckert, J., 2011. Neuropeptide S receptor gene — converging evidence for a role in panic disorder. Mol. Psychiatry 16, 938–948.
Domschke, K., Gajewska, A., Winter, B., Herrmann, M.J., Warrings, B., Mühlberger, A., Wosnitza, K., Glotzbach, E., Conzelmann, A., Dlugos, A., Fobker, M., Jacob, C., Arolt, V., Reif, A., Pauli, P., Zwanzger, P., Deckert, J., 2012a. ADORA2A gene variation,

caffeine, and emotional processing: a multi-level interaction on startle reflex. Neuropsychopharmacology 37, 759–769.
Domschke, K., Klauke, B., Winter, B., Gajewska, A., Herrmann, M.J., Warrings, B., Mühlberger, A., Wosnitza, K., Dlugos, A., Naunin, S., Nienhaus, K., Fobker, M., Jacob, C., Arolt, V., Pauli, P., Reif, A., Zwanzger, P., Deckert, J., 2012b. Modification of caffeine effects on the affect-modulated startle by neuropeptide S receptor gene variation. Psychopharmacology (Berl) 222 (3), 533–541 (Aug).
Donner, J., Haapakoski, R., Ezer, S., Melen, E., Pirkola, S., Gratacos, M., Zucchelli, M., Anedda, F., Johansson, L.E., Söderhäll, C., Orsmark-Pietras, C., Suvisaari, J., Martín-Santos, R., Torrens, M., Silander, K., Terwilliger, J.D., Wickman, M., Pershagen, G., Lönnqvist, J., Peltonen, L., Estivill, X., D'Amato, M., Kere, J., Alenius, H., Hovatta, I., 2010. Assessment of the neuropeptide S system in anxiety disorders. Biol. Psychiatry 68, 474–483.
Dresler, T., Guhn, A., Tupak, S.V., Ehlis, A.C., Herrmann, M.J., Fallgatter, A.J., Deckert, J., Domschke, K., 2013. Revise the revised? New dimensions of the neuroanatomical hypothesis of panic disorder. J. Neural Transm. 120 (1), 3–29 (Jan).
Duangdao, D.M., Clark, S.D., Okamura, N., Reinscheid, R.K., 2009. Behavioral phenotyping of neuropeptide S receptor knockout mice. Behav. Brain Res. 205, 1–9.
Falkenstein, M., 2006. Inhibition, conflict and the Nogo-N2. Clin. Neurophysiol. 117, 1638–1640.
Falkenstein, M., Hohnsbein, J., Hoormann, J., Blanke, L., 1991. Effects of crossmodal divided attention on the ERP components. II. Error processing in choice reaction tasks. Electroencephalogr. Clin. Neurophysiol. 78, 447–455.
Fallgatter, A.J., Ehlis, A.C., Seifert, J., Strik, W.K., Scheuerpflug, P., Zillessen, K.E., Herrmann, M.J., Warnke, A., 2004. Altered response control and anterior cingulate function in attention deficit / hyperactivity disorder boys. Clin. Neurophysiol. 115, 973–981.
Filbey, F.M., Claus, E.D., Morgan, M., Forester, G.R., Hutchison, K., 2012. Dopaminergic genes modulate response inhibition in alcohol abusing adults. Addict. Biol. 17 (6), 1046–1056 (Nov).
Foti, D., Kotov, R., Bromet, E., Hajcak, G., 2012. Beyond the broken error-related negativity: functional and diagnostic correlates of error processing in psychosis. Biol. Psychiatry 71, 864–872.
Gehring, W.J., Fencsik, D.E., 2001. Functions of the medial frontal cortex in the processing of conflict and errors. J. Neurosci. 21, 9430–9437.
Gehring, W.J., Goss, B., Coles, M.G.H., Meyer, D.E., Donchin, E., 1993. A neural system for error detection and compensation. Psychol. Sci. 4, 385–390.
Gorman, J.M., Liebowitz, M.R., Fyer, A.J., Stein, J., 1989. A neuroanatomical hypothesis for panic disorder. Am. J. Psychiatry 146, 148–161.
Gorman, J.M., Kent, J.M., Sullivan, G.M., Coplan, J.D., 2000. Neuroanatomical hypothesis of panic disorder, revised. Am. J. Psychiatry 157, 493–505.
Gottesman, I.I., Gould, T.D., 2003. The endophenotype concept in psychiatry: etymology and strategic intentions. Am. J. Psychiatry 160, 636–645.
Graf, H., Abler, B., Freudenmann, R., Beschoner, P., Scheffeler, E., Spitzer, M., Schab, M., Grön, G., 2011. Neural correlates of error monitoring modulated by atomoxetine in healthy volunteers. Biol. Psychiatry 69, 890–897.
Gratton, G., Coles, M.G., Donchin, E., 1983. A new method for off-line removal of ocular artefact. Electroencephalogr. Clin. Neurophysiol. 55, 468–484.
Gray, J.A., 1982. The Neuropsychology of Anxiety: An Enquiry into the Functions of the Septo-hippocampal System. Oxford University Press, Oxford.
Hajcak, G., McDonald, N., Simons, R.F., 2003. Anxiety and error-related brain activity. Biol. Psychol. 64, 77–90.
Holroyd, C.B., Coles, M.G.H., 2002. The neural basis of human error processing: reinforcement learning, dopamine, and the error-related negativity. Psychol. Rev. 109, 679–709.
Huster, R.J., Westerhausen, R., Pantev, C., Konrad, C., 2010. The role of the cingulate cortex as neural generator of the N200 and P300 in a tactile response inhibition task. Hum. Brain Mapp. 31, 1260–1271.
Jocham, G., Ullsperger, M., 2008. Neuropharmacology of performance monitoring. Neurosci. Biobehav. Rev. 33, 48–60.
Johnson, S.L., Turner, R.J., Iwata, N., 2003. BIS/BAS levels and psychiatric disorder: an epidemiological study. J. Psychopathol. Behav. Assess. 25, 25–36.
Jüngling, K., Seidenbecher, T., Sosulina, L., Lesting, J., Sangha, S., Clark, S.D., Okamura, N., Duangdao, D.M., Xu, Y.L., Reinscheid, R.K., Pape, H.C., 2008. Neuropeptide S-mediated control of fear expression and extinction: role of intercalated GABAergic neurons in the amygdala. Neuron 59, 298–310.
Klauke, B., Deckert, J., Zwanzger, P., Baumann, C., Arolt, V., Pauli, P., Reif, A., Domschke, K., in press. Neuropeptide S receptor gene (NPSR) and life events: G×E effects on anxiety sensitivity and its subdimensions. World J. Biol. Psychiatry. http://dx.doi.org/10.3109/15622975.2011.646302.
Laux, L., Glanzmann, P., Schaffner, P., Spielberger, C.D., 1981. STAI. Das State-Trait-Angst-Inventar: Theoretische Grundlagen und Handweisung. Beltz Testgesellschaft, Weinheim.
McDermott, J.M., Perez-Edgar, K., Henderson, H.A., Chronis-Tuscano, A., Pine, D.S., Fox, N.A., 2009. A history of childhood behavioral inhibition and enhanced response monitoring in adolescence are linked to clinical anxiety. Biol. Psychiatry 65, 445–448.
McNally, R.J., 1996. Anxiety sensitivity is distinguishable from trait anxiety. In: Rapee, R.M. (Ed.), Current Controversies in the Anxiety Disorders. Guilford Press, New York, pp. 214–244.
Mochizuki, T., Kim, J., Sasaki, K., 2010. Microinjection of neuropeptide S into the rat ventral tegmental area induces hyperactivity and increases extracellular levels of dopamine metabolites in the nucleus accumbens shell. Peptides 31, 926–931.
Moran, T.P., Taylor, D., Moser, J.S., 2012. Sex moderates the relationship between worry and performance monitoring brain activity in undergraduates. Int. J. Psychophysiol. 85, 188–194.
Moser, J.S., Schroder, H.S., Heeter, C., Moran, T.P., Lee, Y.H., 2011. Mind your errors: evidence for a neural mechanisms linking growth mind-set to adaptive posterror adjustments. Psychol. Sci. 22, 1484–1489.
Moser, J.S., Moran, T.P., Jendrusina, A.A., 2012. Parsing relationships between dimensions of anxiety and action monitoring brain potentials in female undergraduates. Psychophysiology 49, 3–10.
Nieuwenhuis, S., Ridderinkhof, K.R., Blom, J., Band, G.P., Kok, A., 2001. Error-related brain potentials are differentially related to awareness of response errors: evidence from an antisacccade task. Psychophysiology 38, 752–760.
Nieuwenhuis, S., Yeung, N., van den Wildenberg, W., Ridderinkhof, K.R., 2003. Electrophysiological correlates of anterior cingulate function in a go/no-go task: effects of response conflict and trial type frequency. Cogn. Affect. Behav. Neurosci. 3, 17–26.
Notebaert, W., Houtman, F., Van Opstal, F., Gevers, W., Fias, W., Verguts, T., 2009. Post-error slowing: an orienting account. Cognition 111, 275–279.
Okamura, N., Reinscheid, R.K., 2007. Neuropeptide S: a novel modulator of stress and arousal. Stress 10, 221–226.
Okamura, N., Hashimoto, K., Iyo, M., Shimizu, E., Dempfle, A., Friedel, S., Reinscheid, R.K., 2007. Gender-specific association of a functional coding polymorphism in the Neuropeptide S receptor gene with panic disorder but not with schizophrenia or attention-deficit/hyperactivity disorder. Prog. Neuropsychopharmacol. Biol. Psychiatry 31, 1444–1448.
Olvet, D.M., Hajcak, G., 2008. The error-related negativity (ERN) and psychopathology: toward an endophenotype. Clin. Psychol. Rev. 28, 1343–1354.
Olvet, D.M., Hajcak, G., 2012. The error-related negativity relates to sadness following mood induction among individuals with high neuroticism. Soc. Cogn. Affect. Neurosci. 7, 289–295.
Overbeeck, T.M., Nieuwenhuis, S., Ridderinkhof, K.R., 2005. Dissociable components of error processing: on the functional significance of the Pe vis-a-vis the ERN/Ne. J. Psychophysiol. 19, 319–329.
Pailing, P.E., Segalowitz, S.J., 2004. The effects of uncertainty in error monitoring on associated ERPs. Brain Cogn. 56, 215–233.
Pape, H.C., Jungling, K., Seidenbecher, T., Lesting, J., Reinscheid, R.K., 2010. Neuropeptide S: a transmitter system in the brain regulating fear and anxiety. Neuropharmacology 58, 29–34.
Peterson, R.A., Reiss, S., 1992. Anxiety Sensitivity Index Manual. International Diagnostic Systems, Worthington, OH.
Pourtois, G., Vocat, R., N'diaye, K., Spinelli, L., Seeck, M., Vuilleumier, P., 2010. Errors recruit both cognitive and emotional monitoring systems: simultaneous intracranial recordings in the dorsal anterior cingulate gyrus and amygdala combined with fMRI. Neuropsychologia 48, 1144–1159.
Preacher, K., Hayes, A., 2004. SPSS and SAS procedures for estimating indirect effects in simple mediation models. Behav. Res. Methods Intrum. Comput. 36, 717–731.
Rabbitt, P.M., 1966. Error and error correction in choice-response tasks. Nature 212, 438.
Raiteri, L., Luccini, E., Romei, C., Salvadori, S., Calò, G., 2009. Neuropeptide S selectively inhibits the release of 5-HT and noradrenaline from mouse frontal cortex nerve endings. Br. J. Pharmacol. 157, 474–481.
Rector, N.A., Szacun-Shimizu, K., Leybman, M., 2007. Anxiety sensitivity within the anxiety disorders: disorder-specific sensitivities and depression comorbidity. Behav. Res. Ther. 45, 1967–1975.
Reinscheid, R.K., Xu, Y.L., 2005. Neuropeptide S as a novel arousal promoting peptide transmitter. FEBS J. 272, 5689–5693.
Reinscheid, R.K., Xu, Y.L., Okamura, N., Zeng, J., Chung, S., Pai, R., Wang, Z., Civelli, O., 2005. Pharmacological characterization of human and murine neuropeptide s receptor variants. J. Pharmacol. Exp. Ther. 315, 1338–1345.
Reiss, S., 1997. Trait anxiety: it's not what you think it is. J. Anxiety Disord. 11, 201–214.
Reiss, S., Peterson, R.A., Gursky, D.M., McNally, R.J., 1986. Anxiety sensitivity, anxiety frequency and the prediction of fearfulness. Behav. Res. Ther. 24, 1–8.
Ridderinkhof, K.R., Ullsperger, M., Crone, E.A., Nieuwenhuis, S., 2004a. The role of the medial frontal cortex in cognitive control. Science 306, 443–447.
Ridderinkhof, K.R., van den Wildenberg, W.P., Segalowitz, S.J., Carter, C.S., 2004b. Neurocognitive mechanisms of cognitive control: the role of prefrontal cortex in action selection, response inhibition, performance monitoring, and reward-based learning. Brain Cogn. 56, 129–140.
Ridderinkhof, K.R., Ramautar, J.R., Wjinen, J.G., 2009. To P(E) or not to P(E): a P3-like ERP component reflecting the processing of response errors. Psychophysiology 46, 531–538.
Riesel, A., Weinberg, A., Endrass, T., Kathmann, N., Hajcak, G., 2012. Punishment has a lasting impact on error-related brain activity. Psychophysiology 49, 239–247.
Rizzi, A., Vergura, R., Marzola, G., Ruzza, C., Guerrini, R., Salvadori, S., Regoli, D., Calo, G., 2008. Neuropeptide S is a stimulatory anxiolytic agent: a behavioural study in mice. Br. J. Pharmacol. 154, 471–479.
Roche, R.A., Garavan, H., Foxe, J.J., O'Mara, S.M., 2005. Individual differences discriminate event-related potentials but not performance during response inhibition. Exp. Brain Res. 160, 60–70.
Schmajuk, M., Liotti, M., Busse, L., Woldorff, M.G., 2006. Electrophysiological activity underlying inhibitory control processes in normal adults. Neuropsychologia 44, 384–395.
Schmidt, N.B., Zvolensky, M.J., Maner, J.K., 2006. Anxiety sensitivity: prospective prediction of panic attacks and Axis I pathology. J. Psychiatr. Res. 40, 691–699.
Sehlmeyer, C., Konrad, C., Zwitserlood, P., Arolt, V., Falkenstein, M., Beste, C., 2010. ERP indices for response inhibition are related to anxiety-related personality traits. Neuropsychologia 48, 2488–2495.
Si, W., Aluisio, L., Okamura, N., Clark, S.D., Fraser, I., Sutton, S.W., Bonaventure, P., Reinscheid, R.K., 2010. Neuropeptide S stimulates dopaminergic neurotransmission in the medial prefrontal cortex. J. Neurochem. 115, 475–482.
Smoller, J.W., Tsuang, M.T., 1998. Panic and phobic anxiety: defining phenotypes for genetic studies. Am. J. Psychiatry 155, 1152–1162.
Smoller, J.W., Rosenbaum, J.F., Biederman, J., Susswein, L.S., Kennedy, J., Kagan, J., Snidman, N., Laird, N., Tsuang, M.T., Faraone, S.V., Schwarz, A., Slaugenhaupt, S.A., 2001. Genetic association analysis of behavioral inhibition using candidate loci from mouse models. Am. J. Med. Genet. 105, 226–235.

Sobel, M.E., 1982. Asymptotic confidence intervals for indirect effects in structural equation models. In: Leinhart, S. (Ed.), Sociological Methodology. Jossey-Bass, San Francisco, pp. 290–312.
Spielberger, C.D., Gorsuch, R.L., Lushene, R.E., 1970. Manual for the State-Trait Anxiety Inventory. Consulting Psychologists Press, Palo Alto, CA.
Steinhauser, M., Yeung, N., 2010. Decision processes in human performance monitoring. J. Neurosci. 30, 15643–15653.
Stokes, P.R., Rhodes, R.A., Grasby, P.M., Mehta, M.A., 2011. The effects of the COMT Val108/158Met polymorphism on BOLD activation during working memory, planning, and response inhibition: a role for the posterior cingulate cortex? Neuropsychopharmacology 36, 763–771.
Taylor, S., Koch, W.J., Crockett, D.J., 1991. Anxiety sensitivity, trait anxiety, and the anxiety disorders. J. Anxiety Disord. 5, 293–311.
Taylor, S., Koch, W.J., McNally, R.J., Crockett, D.J., 1992. Conceptualizations of anxiety sensitivity. Psychol. Assess. 4, 245–250.
Ullsperger, M., Harsay, H.A., Wessel, J.R., Ridderinkhof, K.R., 2010. conscious perception of errors and its relation to the anterior insula. Brain Struct. Funct. 214, 629–643.
Vaidyanathan, U., Nelson, L.D., Patrick, C.J., 2012. Clarifying domains of internalizing psychopathology using neurophysiology. Psychol. Med. 42, 447–459.
van Veen, V., Carter, C.S., 2002. The anterior cingulate as a conflict monitor: fMRI and ERP studies. Physiol. Behav. 77, 477–482.
Vocat, R., Pourtois, G., Vuilleumier, P.U., 2008. Unavoidable errors: a spatio-temporal analysis of time-course and neural sources of evoked potentials associated with error processing in a speeded task. Neuropsychologia 46, 2545–2555.
Wegener, G., Finger, B.C., Elfving, B., Keller, K., Liebenberg, N., Fischer, C.W., Singewald, N., Slattery, D.A., Neumann, I.D., Mathé, A.A., in press. Neuropeptide S alters anxiety, but not depression-like behaviour in Flinders Sensitive Line rats: a genetic animal model of depression. Int. J. Neuropsychopharmacol.
Weinberg, A., Olvet, D.M., Hajcak, G., 2010. Increased error-related brain activity in generalized anxiety disorder. Biol. Psychol. 85, 472–480.
Weinberg, A., Klein, D.N., Hajcak, G., 2012. Increased error-related brain activity distinguishes generalized anxiety disorder with and without comorbid major depressive disorder. J. Abnorm. Psychol. 121 (4), 885–896 (Nov).
Willemssen, R., Müller, T., Schwarz, M., Hohnsbein, J., Falkenstein, M., 2008. Error processing in patients with Parkinson's disease: the influence of medication state. J. Neural Transm. 115, 461–468.
Willemssen, R., Müller, T., Schwarz, M., Falkenstein, M., Beste, C., 2009. Response monitoring in de novo patients with Parkinson's disease. PLoS One 4, e4898.
Xiao, Z., Wang, J., Zhang, M., Li, H., Tang, Y., Wang, Y., Fan, Q., Fromson, J.A., 2011. Error-related negativity abnormalities in generalized anxiety disorder and obsessive–compulsive disorder. Prog. Neuropsychopharmacol. Biol. Psychiatry 35, 265–272.
Xu, Y.L., Reinscheid, R.K., Huitron-Resendiz, S., Clark, S.D., Wang, Z., Lin, S.H., Brucher, F.A., Zeng, J., Ly, N.K., Henriksen, S.J., de Lecea, L., Civelli, O., 2004. Neuropeptide S: a neuropeptide promoting arousal and anxiolytic-like effects. Neuron 43, 487–497.
Xu, Y.L., Gall, C.M., Jackson, V.R., Civelli, O., Reinscheid, R.K., 2007. Distribution of neuropeptide S receptor mRNA and neurochemical characteristics of neuropeptide S-expressing neurons in the rat brain. J. Comp. Neurol. 500, 84–201.