Neural correlates of individual differences in anxiety sensitivity: an fMRI study using semantic priming

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Abstract

Individuals with high anxiety sensitivity (AS) have an increased risk of developing anxiety disorders and are more biased in how they process fear-related stimuli. This study investigates the neural correlates of fear-related words and word associations in high- and low-AS individuals. We used a semantic priming paradigm during functional magnetic resonance imaging in which three types of target words (fear symptoms, e.g. ‘dizziness’; neutral, e.g. ‘drink’; and pseudowords, e.g. ‘salkom’) were preceded by two types of prime words (fear-triggers, e.g. ‘elevator’; and neutral, e.g. ‘bottle’). Subjects with high AS rated fear-symptom words (vs neutral words) as more unpleasant than low-AS individuals; they also related these words more strongly to fear-triggers and showed prolonged reaction times. During the processing of fear-symptom words, greater activation in the left anterior insula was observed in high-AS subjects than in low-AS subjects. Lower activation in the left inferior frontal gyrus, angular gyrus, fusiform gyrus and bilateral amygdalae was found in high-AS subjects when fear-symptom words were preceded by fear-trigger words. The findings suggest that cognitive biases and the anterior insula play a crucial role in high-AS individuals. Furthermore, semantic processes may contribute to high AS and the risk of developing anxiety disorders.

Key words: anxiety sensitivity; semantic priming; anterior insula; left inferior prefrontal gyrus; amygdala
Introduction

Individuals with high anxiety sensitivity (AS) are characterized by their fear of anxiety-related sensations (e.g. increased heart rate; Reiss et al., 1986). High AS is therefore considered to be a vulnerability factor for anxiety disorders, particularly panic disorder (PD) (McNally, 2002; Olatunji and Woltzky-Taylor, 2009; Domschke et al., 2010). In non-clinical populations, individuals with high AS are more likely to have a personal and/or family history of panic attacks than those with medium or low AS (Donnell and McNally, 1990). Furthermore, the level of AS predicts the development of spontaneous panic attacks (Schmidt et al., 1999; Plehn and Peterson, 2002; Schmidt et al., 2006).

Previous behavioral research on healthy subjects with high AS has revealed cognitive biases favoring the processing of bodily sensations. High-AS subjects interpret their somatic symptoms in a catastrophic way (interpretive bias: McNally et al., 1999; Teachman, 2005) and give rapid and automatic attention to words associated with anxiety symptoms (attentional bias: Keogh et al., 2001; Hunt et al., 2006; Bar-Haim et al., 2007). These cognitive biases can be related to increased panic attacks and potentially contribute to the development and maintenance of PD in high-AS individuals.

Previous magnetic resonance imaging (MRI) studies of individuals with high AS provide converging evidence for structural and functional alterations to the anterior insula. In functional MRI (fMRI) studies, healthy subjects with high AS showed enhanced activation of the anterior insula during the anticipation of hyperventilation (Holtz et al., 2012) and during the processing of masked fearful faces (Killgore et al., 2011). In patients with anxiety disorders, AS was positively correlated with the thickness and volume of the right anterior insula and activation in the bilateral insulae during the processing of emotional faces (Rosso et al., 2010; Poletti et al., 2015). These findings are plausible considering that the anterior insula is a key node for interoceptive perception (Critchley et al., 2004; Paulus and Stein, 2006; Domschke et al., 2010). The anterior insula also represents a crucial node in the salience network, which is engaged in the detection of and reaction to salient, biologically relevant stimuli (Wiech et al., 2010). Thus, previous findings on the functional and structural alteration of the anterior insula in high-AS individuals indicate that there is a hypersensitivity to interoceptive sensations and/or a hypersensitive salience network in high-AS individuals.

In humans, both somatic symptoms and symbolic representations of threat, such as fear-related words, can activate fear and salience network structures (Lisenberg et al., 1999; Costa et al., 2010). However, the neural correlates of fear-related semantic stimuli processing in high-AS individuals remain unknown. Existing fMRI studies on the processing of emotional words in patients with PD suggest that panic-related words alter the activation of brain regions related to semantic (e.g. left inferior frontal gyrus; IFG), emotion and memory (e.g. amygdala, hippocampus and posterior cingulate) processes (Maddock et al., 2003; van den Heuvel et al., 2005; Dresler et al., 2012). However, it is not known whether this effect is also present in subclinical forms of AS.

Semantic priming is a promising experimental approach for exploring the biased processing of fear-related words and word associations. Semantic priming refers to the improvement in response speed to a target word when it is preceded by a semantically related prime word (e.g. cat-dog) compared to when it is preceded by an unrelated word (e.g. table-dog) (Meyer and Schvaneveldt, 1971). Memory or interpretative biases can be quantified by the priming effect since the semantic activation of one concept in the semantic memory spreads automatically to related concepts, facilitating the retrieval of those concepts (spreading activation model; Collins and Loftus, 1975; Anderson, 1983). The semantic priming paradigm has been used to show that patients with PD demonstrate catastrophic interpretation of bodily symptoms (e.g. Richards and French, 1992; Schneider and Schulte, 2007). fMRI studies have shown that semantic priming effects in healthy subjects result in less attentional demand as well as the alleviation of semantic processing, with a corresponding modulation of activation in the anterior cingulate cortex (ACC), the left IFG and the left temporal cortex during the processing of neutral related and unrelated word pairs (Henson, 2003;Kircher et al., 2009; Sachs et al., 2011). Using semantic priming with positive, negative and neutral related word pairs, activation suppression of the amygdala and enhancement in the right dorsolateral prefrontal cortex (dPFC) have been demonstrated for processing positive related stimuli in patients with depression (Sass et al., 2014a). However, to date, semantic priming has not been used to investigate the neural correlates of the biased processing of fear-related words in high-AS individuals.

To investigate the neural correlates of the symbolic representation of somatic symptoms and potentially biased semantic associations in high-AS individuals, we developed a fear-specific semantic priming paradigm for use in the fMRI scanner. In this paradigm, we presented fear-symptom words (e.g. ‘dizziness’ and ‘sweating’) preceded by neutral words (e.g. ‘window’) or potential fear-trigger words (e.g. ‘elevator’). For the processing of fear-symptom words, we hypothesized that compared to those with low AS, high-AS individuals would (i) rate fear-symptom words (vs neutral words) as more unpleasant, (ii) demonstrate an attentional bias reflected in prolonged response times for the processing of fear-symptom words (vs neutral words) and (iii) show greater activation of the anterior insula and semantic-related brain regions during processing of fear-symptom words (vs neutral words). For the processing of fear-trigger and fear-symptom word associations, we hypothesized that compared to those with low AS, high-AS individuals would (i) perceive greater semantic association between fear-trigger and fear-symptom words, (ii) respond faster to fear-symptom words when they were preceded by fear-trigger words (vs neutral words) and (iii) show modulated activation in brain areas related to semantic priming such as the ACC, left IFG and left temporal cortex as well as in brain regions relevant for the processing of negative emotions (e.g. amygdala, insula).

Methods

Participants

High- and low-AS groups (n = 39 each) were selected from quality-controlled data sets containing 97 right-handed healthy subjects. These data sets were developed by five German centers as part of the national research initiative Panic-Net. AS was measured using the Anxiety Sensitivity Index (ASI; Reiss et al., 1986; German version: Alpers and Pauli, 2001), a 16-item questionnaire in which respondents indicated the degree to which they feared the negative consequences of anxiety on a five-point scale (from 0, ‘very little,’ to 4, ‘very much’). The subjects had ASI scores ranging from 0 to 29, with a median of 8.5. Low (ASI ≤ 8) and high (ASI ≥ 9) AS groups were created by using the median as the cutoff. We then matched the two groups of subjects according to center, gender and age (±5 years).
resulted in the exclusion of a further 19 subjects who could not be matched (see S1 in Supplementary Material). The sociodemographic and psychometric characteristics of the final sample are shown in Table 1. The study was approved by the ethics committees of all participating universities. All subjects gave written, informed consent before participating in the study.

Semantic priming paradigm and stimuli

Each trial started with an attention cue ‘*’ (duration 500 ms) followed by the prime word (duration 200 ms). The target word then appeared for 1000 ms, followed by a number sign (see Figure 1). Immediately after a target word was presented, a lexical decision task was performed. Participants were instructed to press one of two buttons as quickly and accurately as possible in response to the target word (real word—right button; pseudoword—left button). Buttons were pressed using the left index or middle finger in order to avoid motor-related activation in the left hemisphere.

We used two groups of prime words (30 neutral words [N] e.g. ‘window’; 30 potential fear-trigger words [T], e.g. ‘elevator’) and three groups of target words (30 neutral words [N] e.g. ‘curtain’; 30 fear-symptom words [S] e.g. ‘dizziness’; 60 pseudowords [P] e.g. ‘sakom’). All of the real words were nouns. Six conditions were constructed by matching the primes and targets: N–N (neutral-related); T–N (unrelated); N–S (unrelated); T–S (fear-related); and two pseudoword conditions, N–P and T–P (see Table 2). Each condition contained 30 distinct word pairs, or trials. To improve the generalizability of the results and their applicability in clinical trials, we developed two sets of stimuli with comparable word pairs based on previous paradigms (Sass et al., 2014b for a detailed description of experimental stimuli see S2 in Supplementary Material).

Design and procedure

We used a rapid event-related fMRI design that has been successfully applied in a number of our previous studies (Kircher et al., 2014a). Small blocks of stimuli containing one to three trials from the same condition were constructed, with a small intertrial interval (ITI) of 1.5–2.5 s (M ITIsmall = 2 s). Longer ITIs (jittered 3–5 s; M ITIlong = 4 s) were placed randomly between the small blocks so that the overlapping hemodynamic response functions (HRF) could be deconvolved (Sass et al., 2009). Four pseudo-randomized versions of the experiment were counterbalanced across subjects to avoid a systematic effect of condition order. The stimuli display was controlled using a Presentation script file (Version 11.0, software package, Neurobehavioral Systems).

Following the fMRI session, subjects rated the valence (from -3, ‘highly negative,’ to 3, ‘highly positive’) and relatedness (from 1, ‘unrelated,’ to 7 ‘highly related’) of the word pairs where the target was a real word.

fMRI data acquisition

fMRI brain images were acquired using 3T MRI scanners in each center. A total of 435 transversal functional images (echo-planar images, 64 × 64 matrix; 30 slices ascending; field of view [FoV] = 230 mm; repetition time [TR] = 2000 ms; echo time [TE] = 30 ms; flip angle = 90°; slice thickness = 3.8 mm; voxel resolution = 3.6 × 3.6 mm) that covered the whole brain and were positioned parallel to the intercommissural line (anterior commissure-posterior commissure) were recorded. Routine quality control measures were carried out to ensure a high standard of fMRI data acquisition and data quality (for scanner specifications and quality control see S1 in Supplementary Material).

Behavioral data analysis

Reaction time (RT) was calculated for correct responses only. To reduce errors, individual RTs were eliminated if they exceeded the mean of their condition by more than two standard deviations (Ratcliff, 1993). RTs, valence and relatedness ratings were entered into three separate repeated-measures analyses of variance (ANOVAs) with the target (fear-symptom and neutral) and

![Fig. 1. Schematic display of the semantic priming task.](image)

Table 1. Social demographical and psychological characteristics of high- and low-AS subjects

|                          | High AS (n = 39) | Low AS (n = 39) | t/Chi² |
|--------------------------|-----------------|-----------------|--------|
| Age in years             | 33.05 ± 11.94   | 32.26 ± 10.89   | 0.31   |
| Female gender            | 25 (64.1%)      | 25 (64.1%)      | 0      |
| Tobacco use              | 1 smokers       | 14 smokers      | 0.53   |
| Years of education       | 1.58            |                 |        |
| ≤8                       | 1               | 1               |        |
| 9–11                     | 4               | 4               |        |
| ≥12                      | 34              | 30              |        |
| Digit span forward       | 8.21 ± 2.22     | 8.46 ± 2.09     | 0.53   |
| Digit span backward      | 7.64 ± 1.80     | 7.74 ± 2.37     | 0.22   |
| TMT-A                    | 24.36 ± 8.04    | 23.92 ± 8.02    | 0.24   |
| TMT-B                    | 52.07 ± 17.61   | 55.76 ± 23.28   | 0.17   |
| RWT-K                    | 10.82 ± 4.33    | 10.67 ± 3.79    | 0.79   |
| RWT-K                    | 13.54 ± 4.05    | 14.28 ± 3.89    | 0.83   |
| ASI                      | 15.72 ± 4.05    | 14.28 ± 3.89    | 0.79   |
| BDI                      | 3.08 ± 3.55     | 1.41 ± 1.83     | 2.61   |

M, mean; SD, standard deviation; TMT, trail making test; RWT, Regensburg word fluency test; ASI, anxiety sensitivity index; BDI, Beck depression inventory.

Table 2. Experimental design of the semantic priming paradigm and examples of stimuli

| Prime / Target | Neutral (N) | Fear symptom (S) | Pseudoword (P) |
|---------------|-------------|------------------|---------------|
| Neutral (N)   | N–N         | N–S              | N–P           |
|               | bottle-drink| bottle-breathless| bottle-sakom  |
| Fear trigger (T)| T–N         | T–S              | T–P           |
|               | elevator-drink| elevator-dizzy | elevator-tuneu|
|               | bus–curtain | bus–breathless  | bus–faussak  |

Note: All real words consisted of German words, here translated to English for illustrative purpose.
prime (fear-trigger and neutral) as the within-subject variables and the group (high- and low-AS) as the between-subject factor. Tukey’s HSD post-hoc tests were applied to evaluate significant differences between groups or conditions.

fMRI data analysis
Magnetic resonance images were analyzed using standard routines of Statistical Parametric Mapping (SPM8; www.fil.ion.ucl.ac.uk) implemented in MATLAB 7.7 (MathWorks, Sherborn, Massachusetts). The first five volumes of each functional run were discarded to minimize T1 saturation effects. For data preprocessing, standard slice-timing (middle slice), realignment and normalizing functions (Montreal Neurological Institute [MNI] template 2 × 2 × 2 mm) of SPM8 were applied. To account for differences in intrinsic smoothness between scanners, an iterative smoothness equalization procedure was performed for all data sets using a target smoothness of 10 mm full width at half maximum Gaussian isotropic kernel (Friedman et al., 2006).

For single subject analyses, realignment parameters were included as regressors of no interest to account for movement artifacts. Low frequencies were removed using a high-pass filter with a cutoff period of 128 s. The hemodynamic response triggered by the target words in each condition was modeled with a canonical HRF. Parameter estimates (b) and t-statistic images were calculated for each subject. Next, we performed a random effects group analysis by entering the parameter estimates for each group (high- and low-AS) into a full factorial analysis. The fMRI centers were introduced as covariates to account for scanner differences. Further covariates of no interest included age, level of education and history of smoking.

To correct for multiple comparisons errors, we employed the Monte Carlo simulation of brain volume to establish an appropriate voxel contiguity threshold (Forman et al., 1995; Slotnick et al., 2003). Assuming a single voxel type-I error of P < 0.005, a cluster extent of 115 contiguous resampled voxels was indicated as necessary to correct for multiple voxel comparisons at P < 0.05.

The reported voxel coordinates of activation peaks correspond to the MNI space (ICBM standard). For anatomical localization, functional data were referenced against probabilistic cytoarchitectonic maps (Eickhoff et al., 2005).

Contrasts of interest. Following our hypotheses on AS, contrasts of interest focused on differences between the high- and low-AS groups. However, we report the general task effects (cross-groups) in S4 and S5 in the Supplementary Material, since they support the validity of our experimental paradigm.

We hypothesized that the high-AS group would demonstrate greater neural activation for fear-symptom word processing than the low-AS group. To test this hypothesis, we performed a directed t-contrast comparing high- to low-AS group in their activation in conditions with fear-symptom words subtracted by the activation in neutral target word conditions (group × target: high AS (symptom > neutral) > low AS (symptom > neutral)).

We were also interested in group differences in the modulatory effects of fear-trigger words for the processing of (i) neutral target words and (ii) fear-symptom target words, as these could lead to response suppression or enhancement during the processing of target words. As such, we used F-contrasts (without directionality; see also Sass et al., 2012) to test our hypothesis of group differences in the neural processing of word-pair associations. Using F-contrasts, we compared how high- and low-AS subjects differed in how they processed N–N and T–N conditions (group × prime: high AS [N–N vs T–N] vs low AS [N–N vs T–N]) and T–S and N–S conditions (group × prime: high AS [N–S vs T–S] vs low AS [N–S vs T–S]).

For all significant clusters in the F-contrasts, we provided additional information about the direction of the effect (by applying post-hoc t-contrasts) and the group specificity of the modulation effects (by testing the significance of ‘group × target × prime’ using repeated-measures ANOVAs; see Table 4). The ANOVAs were calculated based on extracted individual parameter estimates (using the VOI function of SPM8) for each cluster for the four experimental conditions.

The cluster threshold procedure meant that there was the potential to miss effects in small brain structures such as amygdala. Therefore, we carried out region-of-interest (ROI) analyses for the bilateral amygdalae (defined by SPM toolbox WFU PickAtlas) on group differences in the target effects and modulation effects of fear-trigger words to fear-symptom words. The statistical parametric maps used in the ROI analyses were thresholded at P < 0.005 at the voxel level. This was combined with a cluster extent threshold of P < 0.05, corrected for multiple comparisons using the FWE correction implemented in SPM8. The same ANOVAs were also performed for clusters in the amygdalae.

Results
Behavioral and ratings data
RTs in the lexical decision task. Both the high- and low-AS groups made few errors (with a maximum mean error of 4.0%) in the lexical decision task, and there were no group differences in terms of accuracy. A repeated-measures ANOVA of RTs yielded significant main effects for target (F(1,76) = 128.85, P < 0.001), with slower responses to fear-symptom words than neutral words. The same was true for prime (F(1,76) = 8.45, P < 0.01), with prolonged RTs for neutral or fear-symptom target words preceded by fear-trigger words. The significant target × prime interaction (F(1,76) = 10.17, P < 0.01) and the subsequent post-hoc test results suggest that there was a priming effect in N–N (N–N < T–N, P < 0.01) but not in T–S.

The significant group × target interaction (F(1,76) = 4.39, P < 0.05) indicated a larger target effect for the high-AS group than for the low-AS group. Despite the tendency for high-AS subjects to respond more quickly to neutral target words and more slowly to fear-symptom words than low-AS subjects, post-hoc tests revealed no significant differences in RTs between the two groups. No significant interactions were observed for group × prime (P = 0.52) or group × target × prime (P = 0.17). An overview of behavioral performance is given in Table 3.

Ratings data. In the valence ratings we found significant main effects for target (F(1,70) = 98.92, P < 0.001) and prime (F(1,70) = 35.39, P < 0.001), indicating that fear-symptom target words and fear-trigger prime words were more unpleasant than neutral words for both high- and low-AS subjects. Among the interaction effects, only group × target reached marginal significance in the ANOVA (group × target: F(1,70) = 3.56, P = 0.06). However, the tendency for high-AS subjects to rate word pairs containing fear symptoms as more unpleasant than low-AS subjects (high AS: M = −0.47, SD = 0.53; low AS: M = −0.27, SD = 0.42) was statistically not significant in the post-hoc test (see S3.1 in the Supplementary Material).
In the relatedness ratings we observed significant main effects for target \(F(1,70) = 1023.71, P < 0.001\) and prime \(F(1,70) = 1582.15, P < 0.001\). However, both main effects were predominately driven by the very high relatedness rating of N–N. Furthermore, we obtained significant interactions for group \(\times\) target \(F(1,70) = 9.77, P < 0.01\) and group \(\times\) prime \(F(1,70) = 6.45, P < 0.05\). Post-hoc tests indicated that compared to those with low AS, high-AS subjects perceived significantly higher relatedness of word pairs containing fear-trigger words \((P < 0.01)\) or fear-symptom words \((P < 0.01)\); however, the same was not true for word pairs containing neutral words (see S3.2 in the Supplementary Material).

**Imaging data**

**Target effect (fear symptom > neutral target).** For the processing of fear-symptom words (symptom > neutral) we found activation across all subjects within the left hemispheric semantic network (e.g. IFG, middle temporal gyrus) and brain regions related to fear processing (e.g. amygdala, insula, thalamus; see S4 in Supplementary Material). Group differences in terms of the target-word effect were confined to the left insula with a small extension into the putamen and left IFG (see Figure 2). The contrast estimates (extracted using the VOI function of SPM8) during the processing of fear-symptom words subtracted by the neutral target-word processing (fear-symptom words > neutral target words) were positively correlated with the ASI score only in the high-AS group \((r = 0.42, P < 0.01\); see Figure 2) and were significantly higher than the correlation coefficient in the low-AS group \((r = 0.01, P = 0.94; z = 1.86, P_{one-tailed} = 0.03\).

**Modulation effect of fear-trigger words to neutral target words (N–N vs T–N).** As shown in the Supplementary material, activation differences in the F-contrast \((N–N \text{ vs } T–N)\) between the high- and low-AS groups were found in semantic priming-related cortices, such as the left IFG, superior frontal gyrus and left supramarginal gyrus (see S5.1 in Supplementary Material). No group differences were detected in the modulation of neutral target words.

**Modulation effect of fear-trigger words to fear-symptom words (N–S vs T–S).** The group differences in this modulation effect were very prominent along the left IFG (BA 44) and angular gyrus (BA 39). Another significant cluster was observed in the right fusiform gyrus (see Figure 3 and Table 4). T-contrasts in all three clusters indicated lower activation in high-AS subjects if fear-symptom words were preceded by potential fear-trigger words \((T–S < N–S; \text{ see } ‘Direction of modulation’ \text{ in Table } 4)\). Low-AS subjects showed stronger activation in the T–S condition. The group \(\times\) target \(\times\) prime interaction was significant in the left angular gyrus \((F(1,76) = 9.62, P < 0.01)\) and right fusiform gyrus \((F(1,76) = 6.45, P < 0.05; \text{ see } ‘\text{Table } 4)\), while the interaction effect in the left IFG reached marginal significance \((F(1,76) = 3.13, P = 0.08)\). However, the left IFG, angular gyrus and right fusiform gyrus showed significant group \(\times\) prime interactions \((IFG: F(1,76) = 8.68, P < 0.01; \text{ angular gyrus: } F(1,76) = 4.65, P < 0.05; \text{ fusiform gyrus: } F(1,76) = 10.21, P < 0.01; \text{ see } \dagger \text{ in Table } 4)\), suggesting a general activation suppression in conditions containing fear-
trigger prime words (vs neutral prime words) in the high-AS group (vs the low-AS group).

**ROI analyses for amygdala.** In the ROI analysis, significant group differences were not detected for target effects. However, differences were found in the modulation effect of fear-trigger words to fear-symptom words (high AS (N–S > T–S) > low AS (N–S > T–S)). We found that the activation of the bilateral amygdala in high-AS subjects was selectively higher for the processing of N–S word pairs and lower for the processing of T–S word pairs. By contrast, in low-AS subjects, the amygdala was more activated in the T–S condition than in the N–S condition (see Figure 4). We found a significant group × target × prime interaction in the left amygdala (F(1,76) = 4.35, P < 0.05) and a group × prime interaction in the left and right amygdala (Left: F(1,76) = 7.01, P < 0.01; Right: F(1,76) = 6.43, P < 0.01). Compared to those with low AS, high-AS subjects showed specific modulation effects (activation suppression) in the left amygdala for the processing of fear-symptom words preceded by fear-trigger words. High-AS subjects generally demonstrated less activation in the bilateral amygdalae in conditions containing fear-trigger prime words (vs neutral words) than those with low AS.

**Discussion**

This study investigated the neural correlates of individual differences in AS using a semantic priming task. The findings supported our hypothesis of biased processing of fear-symptom words in high-AS subjects. Compared to those with low AS, these individuals rated fear-symptom words (vs neutral words) as more unpleasant, demonstrated prolonged reaction times for processing fear-symptom words (vs neutral words), and showed greater activation of the left anterior insula during the processing of fear-symptom words (vs neutral words). This last finding also correlated positively with the severity of AS exhibited by high-AS subjects, as measured by the Anxiety Sensitivity Index. High-AS subjects also demonstrated altered neural processing of fear-symptom words preceded by potential fear-trigger words, suggesting a biased semantic network structure in high-AS individuals. Despite the lack of a behavioral priming effect, high-AS subjects explicitly reported a higher degree of relatedness between fear-trigger/fear-symptom word pairs and showed less activation than low-AS individuals in the left IFG, angular gyrus and amygdala while implicitly processing fear-symptom words preceded by fear-trigger words (vs neutral prime words). This lower activity might reflect a facilitation of semantic processing and an inhibition of emotional processing of the fear-symptom target words that followed.

We found significant group × target interactions in valence ratings, the lexical decision task and neural activity. This is in line with our first hypothesis regarding biased fear-symptom word processing in high-AS subjects compared to those with low AS. Compared to low-AS subjects, there was greater left anterior insula activation in high-AS individuals for the processing of fear-symptom words as well as a positive correlation between AS and the involvement of the left anterior insula. In line with previous structural and functional MRI studies on AS (Rosso et al., 2010; Killgore et al., 2011; Holtz et al., 2012; Poletti et al., 2015), our findings lend strong support to the hypothesis that the insula plays a crucial role in individuals prone to anxiety (Paulus and Stein, 2006). In our study, fear-symptom words described somatic fear symptoms, such as ‘increased heart rate,’ ‘sweating,’ and ‘dizziness.’ It has been proposed that these somatic sensations are integrated with the anterior insula, a key cortical structure involved in interoceptive perception (Craig, 2002; Critchley et al., 2004). Bodily sensations correlate with activation in the anterior insula (Craig, 2011; Oosterwijk et al., 2012). As such, our findings could be interpreted as showing that high-AS individuals demonstrate stronger interoceptive perception during the processing of fear-symptom words. Since the anterior insula is a key structure in the salience network (Wiech et al., 2010), our data could also suggest that fear-symptom words—as signals of potentially harmful or undesirable outcomes—could be perceived as more salient by high-AS individuals and consequently lead to stronger arousal. Future studies with psychophysiological measurements (e.g. heart rate and skin conductance) might clarify the contribution of bodily responses to anterior insula activity during fear-related word processing. Nevertheless, the left anterior insula is also involved in broad cognitive functions, such as language processing (Ackermann and Riecker, 2010; Oh et al., 2014). The left anterior insula activation in high-AS subjects in the current study had cluster extensions into the neighboring IFG and putamen. We speculate that the left anterior insula and its surrounding structures could function as a platform for the interaction between semantic and fear processing. This is one explanation for the difference in the laterality of our results and those in the study of Killgore et al. (2011), where the activation of the right anterior

### Table 4. Neural correlates of group differences in modulation effects of fear-trigger words

| Coordinates | t-value |
|-------------|---------|
| Anatomical region BA | x | y | z | F-value | no. voxels | Direction of modulation |
| **Group difference of modulation effect to neutral target words** (N–S vs T–N × high AS vs low AS) | | | | | | No cluster exceed the significance test |
| **Group difference of modulation effect to fear-symptom words** (N–S vs T–S × high AS vs low AS) | | | | | | Left IFG 44 | –48 | 34 | 16 | 13.34† | 325 | High AS (N–S > T–S) > Low AS (N–S > T–S) |
| | | | | | | Right fusiform gyrus 20 | 38 | –34 | –24 | 16.73†  | 129 | High AS (N–S > T–S) > Low AS (N–S > T–S) |
| | | | | | | Left angular gyrus 39 | –36 | –68 | 42 | 14.85†  | 126 | High AS (N–S > T–S) > Low AS (N–S > T–S) |

Coordinates are listed in MNI space. Significance level: uncorrected P < 0.005, cluster with at least 115 voxels. Directions of modulation were determined by post-hoc t-tests within the clusters which were significant in the F-contrasts.* and † represent the significance (P < 0.05) of group differences in interaction effects between target and prime (group × target × prime) and prime effects (group × prime) respectively using repeated measure ANOVAs with the parameter estimates in the corresponding clusters. AS: anxiety sensitivity; IFG: inferior frontal gyrus.
insula was correlated with AS during the processing of fearful faces.

We also found group differences in relatedness ratings and the neural processing of fear-trigger/fear-symptom word pairs. The stronger relatedness for fear-trigger/fear-symptom word pairs perceived by high-AS (vs low-AS) subjects suggests an enhanced connection between these fear concepts (according to the associative network model of emotion; Lang, 1979; Lang et al., 2000) and a covariation bias for fear triggers and fear symptoms in high-AS individuals (Tomarken et al., 1989). This enhanced explicitly evaluated association in high-AS subjects most likely alters the implicit processing of fear-symptom words when they are preceded by fear-trigger words. Although this effect could not be demonstrated in RTs, less activation in the left IFG (BA 44) and angular gyrus (BA 39) in high-AS subjects may indicate a facilitation of the semantic processing of fear-symptom words in the ‘fear-related’ (T–S) condition. Various studies show that the left IFG and angular gyrus are often activated in semantic processing tasks (Thompson-Schill et al., 1997; Bookheimer, 2002; Vigneau et al., 2006; Binder et al., 2009).

In high-AS subjects, we suggest that the activation of neural networks representing fear-trigger words is likely to spread automatically to neural networks representing fear-symptom words if they are preceded by fear-trigger words. Conversely, if a neutral prime word is followed by a fear-symptom target word, the left posterior IFG is activated more strongly in high-AS subjects. This can be seen as a way of enhanced processing of the fear-symptom word due to its conflict with the neutral prime or as a means of inhibiting the automatic activation of the neural networks representing neutral target words, or perhaps as a combination of both. The left angular gyrus plays a role in supramodal concept retrieval and conceptual integration (Vigneau et al., 2006; Binder et al., 2009), where semantic associations are elaborated (Wagner et al., 2001). In high-AS subjects,
the lower activation in the left angular gyrus during T–S processing (vs N–S processing) suggests relative ease in how these individuals integrate the concepts of fear-trigger and fear-symptom words. We also found less activation of the bilateral amygdalae in high-AS subjects in the ‘fear-related’ (T–S) condition. Since the amygdala plays a crucial role in the recognition and perception of fear (Phelps et al., 2001; Feinstein et al., 2011; Adolphs, 2013), less activation in this structure in high-AS subjects could reflect successful inhibition of the fear reaction through the priming of potential threats. However, if threats appear unexpectedly (as in the N–S condition), greater amygdala activation was found in high-AS subjects, suggesting a stronger fear response. This result is in line with evidence that activation in the amygdala is lower if unconditioned aversive stimuli are expected (Belova et al., 2007; Johansen et al., 2010).

Our results provide the first evidence of altered semantic network activity in high-AS subjects. In our study, prime words were presented for only 200 ms. Thus, the influence of prime words on target words could have had very little impact on automatic processes. The activation of fear triggers could, in this case, spread unconsciously to the representation fear responses in high-AS subjects, leading to lower neural activation for the processing of fear-symptom words. Although the high-AS subjects had no history of panic disorder (PD), they demonstrated different evaluation and neural processing of fear-trigger/fear-symptom associations compared to low-AS subjects. This could represent a cognitive vulnerability for high-AS subjects and account for the higher risk of such individuals developing anxiety disorders such as PD.

Besides the modulation effects presented above, we also observed a general lower activation in high-AS subjects (vs low-AS subjects) in the left IFG, angular gyrus, right fusiform gyrus and amygdala during the processing of all target words preceded by fear-trigger prime words (group × prime interaction effect). This effect was most likely due to adequate cognitive processing and suppression in high-AS subjects exposed to fear-trigger stimuli.

Despite these important findings, our study has several important limitations. First, we found no behavioral evidence for a priming effect in the T–S condition (vs N–S). Thus, our findings on a neural level actually represent priming, facilitation, or suppression effects, which appear to be more related to semantic processing (fear-related words and the triggering of emotional reactions) than to the more basic performance in the lexical decision task. Second, subjects were assigned to one of two groups according to their AS scores using a median split. Other studies compare more extreme AS groupings (Melzig et al., 2009). Therefore, it is important to note that differences detected between the groups in our study were fairly subtle. We also used a high number of potential trigger words, which may not all serve as subjective trigger situations for any given subject with high AS.

To summarize, our results suggest that the anterior insula plays a crucial role in AS during the semantic processing of fear-symptom words. Furthermore, the enhanced semantic association between fear-trigger and fear-symptom words in high-AS individuals modulated processing in semantic and fear-related brain regions. Thus, cognitive biases and the corresponding neural correlates are already present in healthy subjects with high AS, and this may contribute to the development of anxiety disorders.

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Supplementary data

Supplementary data are available at SCAN online.

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