**Supplementary Material**

**Supplementary Analyses & Results**

1. **Meta-Analytic decoding of statistical Images**

In order to provide an interpretation of the whole-brain voxel-wise data from the connectivity analysis (both with the amygdala and cognitive emotion regulation network as a seed) we applied a novel method to “decode” these statistical images using Neurosynth (Yarkoni *et al.* 2011). This method essentially correlates an input image with reverse inference maps related to a certain topic (this is a map of the probability per voxel of being active given a certain topic) see Chang *et al.* 2012 for such an approach in decoding the functional subdivision of the Insula. The analysis thus gives an automatic interpretation of the “involvement” of an input image (for instance a specific brain region, or a contrast image of different conditions) in a certain a topic (e.g. ‘emotion’). Since many terms in the Neurosynth database are conceptually overlapping, we aimed to further reduce the number of terms to broader (latent) topics (see (Poldrack *et al.* 2012) for a comparable approach. Here, we initially reduced the number of topics to 40, and then excluded topics related to (fMRI) methods, resulting in 27 topics. For interpretation purposes, these topics were labeled to broadly capture the content of the terms (for example “social cognition”, “emotion”, “working memory”, “language”), see table S1 for a complete list of topic with all associated terms. As input images for the decoding analysis, we used the contrast maps of the speech anticipation compared to the baseline and recovery scans (quadratic effects) of both the amygdala and CER seed, and for both the control and social anxiety group. The correlation values (indicating the association between the input image and the topic) of the decoding analysis were transformed to Fisher *z* scores, and ranked based on differences between the two groups. This approach gives an overview of the involvement of a seed region during speech anticipation with other (regions associated to these) topics. Since the topics are ranked based on group differences, it further provides a qualitative indication (i.e. not tested for statistical significance) of which topics differentiate the two groups most. Results of the meta-analytic decoding analysis are presented in Figure S1

Table S1. List of the different topics and associated terms used in meta-analytic decoding. Only non-method related topics (shaded) were used in the analysis..



Figure S1. Ranked differences between the social anxiety and control groups’ connectivity maps to topics based on meta-analytic brain imaging data. (a) amygdala connectivity (b) cognitive emotion regulation connectivity. Positive values indicate a relative stronger involvement of a topic in the control group, negative values a relative stronger involvement in the social anxiety group.

**2. Mediation analyses**

In order to examine whether changes in brain connectivity might drive the relation between social anxiety symptoms (SPAI-SP) (predictor) and change in self-reported stress (outcome), a mediation analysis was performed (Baron & Kenny 1986). The predictor-outcome relation is referred to as effect *c*, and the direct effect controlling for the mediator as *c’*. Effect *a* is the relation between predictor and mediator, effect b the relation between mediator and outcome, and *a\*b* refers to the mediation, or more general, the indirect effect (see Mathieu & Taylor 2006; Hayes 2009 for a discussion on the differences between mediation and indirect effects). The *a\*b* effect tests the significance of *c – c’*, for which a bootstrapping procedure is applied (Wager *et al.* 2008). The mediation analyses were applied to both the connectivity results from the initial analysis (CER-AMY z values), and the connectivity z-values restricted to the significant amygdala voxels from the voxel-wise regression analysis with the CER time-series as regressor. From these significant voxels in the Group x Run interaction, the times-series was extracted, correlated with the CER time-series and Fisher z transformed. Brain connectivity (difference R2 – R1 from the voxel-wise analysis results) mediated the relationship between social anxiety symptoms and increased stress responses (*a\*b*=0.016, *z=*1.99, *p*=0.047,see figure S2). Path *c* (SPAI-SP – self-reported stress) was trend significant (*c*=0.035, *z=*1.86, *p*=0.065), path *a* was significant (social anxiety symptoms to connectivity, *a*=0.0047, *z=*3.079, *p*=0.0021) but path *b* (brain connectivity to change in reported stress, *b*=3.63, *z=*1.51, *p*=0.13) was not. The significant positive indirect effect, and the positive values of path *a* and *b* (note that we are referring to the direction, not the significance, of the individual paths coefficients) suggest that higher social anxiety symptoms were related to reduced functioning integration of brain connectivity (moving from “negative” connectivity to zero or positive connectivity values), and the greater this change in brain connectivity, the greater the increase in reported stress.

Figure S2. Mediation Results



Path diagram showing the relation between social anxiety symptoms (SPAI-SP), changes in brain connectivity (run2 – run1; based on voxel-wise results, FWE corrected for amygdala) and changes in self-reported stress ratings (run2 – run1). Path *a* is the connection between anxiety symptoms and brain connectivity, path *b* the connection between brain connectivity and perceived stress. Path *c’* is the direct connection between anxiety symptoms and perceived stress controlling for the mediator. The indirect, mediation, path (a\*b) is shown as an arc connecting anxiety symptoms and perceived stress. Values for each path represent beta values with standard error in parentheses, significant paths are bold.

**3. Rank Percentage of activity level.**

Assessing brain activity in resting state fMRI scans is difficult because of a lack of a baseline condition within each scan to compare the activity to. Yet, in order to approximate activity within each scan, the following procedure was applied: for each participant and scan, the pre-processed data was averaged over the entire time-series per voxel. These mean values per voxel were subsequently rank-ordered across the whole brain, and transformed to a percentage (rank order/total voxels \*100). As such, a value per voxel is obtained that describes the relative strength (as a percentage) of intensity compared to the rest of the brain. These values were subsequently averaged per region (left and right amygdala and cortical emotion regulation regions) and entered in a repeated-measures ANOVA, with group as between- and run as within-subjects factor. For the analysis of the amygdala, side (left or right) was additionally entered as within-subjects factor.

For the rank-ordered percentage of the CER, a repeated measures ANOVA with group as between- and run as within-subjects factor, showed a main effect of run (*F*(2,76)=3,77, *p*=0.027). The group x run interaction showed a trend towards a significant effect on the quadratic contrast (*F*(1,38)=3.1, *p*=0.087), see figure S2. Group showed no significant main effect (*F*(1,38)=1.98, *p*=0.16). For the analysis of the amygdala, a repeated measures ANOVA with group as between-, and run and side (left or right) as within-subjects factors. Side showed a main effect (*F*(1,38)=52.24, *p*<0.001), there was no significant effect of run or a run x group interaction (both *p*>0.32). There was no significant main effect of group (*F*(1,38)=1.01, *p*=0.32). Note that the between subject standard error was in a much higher range (ranging from 1.23 to 3.67 rank percentage points) than the within-subject standard error presented in figure S2, explaining the non-significant group differences.

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Figure S3. Rank percentage of activity level.

Normalized levels of intensity for each group and run (R1, baseline, R2, speech anticipation, R3, recovery). (a) Cortical emotion regulation regions (b) left amygdala (c) right amygdala. Error bars represent *within* subject standard error of the mean.

**4. Time-varying changes in heart rate and cortical-amygdala connectivity**

To explore the possibility that connectivity between the amygdala and the cortical emotion regulation regions would vary during the course of the speech anticipation measurement (R2), and to visualize this response, we estimated dynamic changes in connectivity. This analysis was done by applying a Gaussian sliding window of 60 TR over the time-series to calculate the Pearson correlation coefficient, and employing a Fisher *z* transform (see(Chang & Glover 2009) for a similar approach). The analyses were performed on the CER with the left and right amygdala separately. To subsequently test if the two groups differed in onset and duration of this connectivity or heart rate responses during the speech anticipation run, change-point estimation was applied, using hierarchical exponentially weighted moving average (HEWMA) (Lindquist *et al.* 2007). A change point here was defined as a difference between the two groups after the baseline (the last 100 data points of the first run). To correct for multiple comparisons the HEWMA method incorporates a monte-carlo procedure, and tests observed differences between the two groups to a distribution of maximum *t* values from randomly generated time-series of equal length. Figure S3 display the time-varying responses per measurement period. No significant change-points between the groups were observed for any of the three measurements (Heart rate, CER-lAmygdala connectivity, CER-rAmygdala connectivity, all p>0.39).

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Figure S4. Time-Varying Estimates of Heart Rate and Connectivity

Time-varying estimates per measurement period (Run1; baseline, Run2; speech anticipation, and R3; recovery). All values are baseline (R1) corrected. (a) Heart rate (b) Left amygdala – CER connectivity (C) Right amygdala – CER connectivity. Shaded area is standard error of the mean per group. For visualization purposes exponential smoothing was applied: a factor of 0.05 for the heart rate and 0.15 for the amygdala-CER connectivity time-series.

**5. “Confounding” effects in resting-state fMRI**

Recent years have seen an increasing concern that resting-state connectivity results, and especially group differences can be confounded by factors such as head motion (Van Dijk *et al.* 2012) and global signal regression (Murphy *et al.* 2013). With respect to the effect of head motion, it is important to note that in the current analysis the motion parameters were regressed out the preprocessed data, although chances are that residual effects of motion can remain present (Van Dijk *et al.* 2012; Murphy *et al.* 2013). Additionally, it is of note that for the main metrics of confounding motion parameter effects proposed in Van Dijk *et al.* 2012 (mean displacement, maximum displacement, and number of movements), there was no significant group x run interaction (overall or quadratic effect), all *p*>0.1. Global signal regression is an often applied preprocessing step in resting-state fMRI to remove non-neuronal global confounding factors, and is suggested to increase the sensitivity of resting state connectivity (Fox *et al.* 2009; Cole *et al.* 2010), but has also recently been criticized for, among others, inducing negative correlations (Murphy *et al.* 2009; Saad *et al.* 2012). Performing the same analysis without global signal regression does indeed change the sign of the connectivity values (Controls; R1 = 0.28; R2 = 0.24; R3 = 0.36; Social Anxiety; R1 = 0.40; R2 = 0.41; R3 = 0.31) and the CER-amygdala connectivity quadratic run effect x group interaction is trend significant, *F*(1,38)=4.045, *p*=0.051. These finding underscore that our connectivity results should be interpreted as relative connectivity values with respect to global signal fluctuations.

**Supplementary Discussion**

A novel topic mapping approach using the neurosynth database was used to obtain an automatic “interpretation” of the statistical maps of speech anticipatory related changes in amygdala and cognitive emotion regulation connectivity. Broadly, the results indicated that the control group showed relatively less involvement of amygdala connectivity with topics such as “emotion”, “social cognition” and “memory”, and stronger to topics as for instance “perception”. The cognitive emotion regulation results are largely in the opposite direction. The results are generally in line with our notion that the amygdala in the control group is more connected to (and perhaps regulated by) cognition related regions, while in the social anxiety group it is more linked to other emotion and stress related regions. These results provide an interesting additional and, importantly, automatic interpretation of the differences in connectivity patterns between the two groups.

 Although the mediation analysis results show an interesting indirect effect of cortical amygdala connectivity, social anxiety symptoms and current stress levels during speech anticipation, it does have to be noted that only the mediation results from the time-series connectivity extracted from the voxel-wise analysis (amygdala voxels) were significant. The connectivity results from the initial analysis (whole amygdala) were not significant, although the coefficients of each of the paths were in the same direction. It is possible that this is simply a result of a lack of statistical power (i.e. with a larger subject sample the results would have been significant for the whole amygdala time-series). Another possibility is that only part of the amygdala (here, maybe the basolateral nuclei) is important for the mediation effect. We do note however, that our current method of extracting one time-series over a broad set of cortical regions, in combination with the inherent problem in fMRI to accurately differentiate between activities in small adjacent structures, makes inference about subnuclei functioning of the amygdala extremely difficult.

Amygdala connectivity has been linked to anxiety disorders (Kim *et al.* 2011) and our data emphasizes the importance of amygdala connectivity for SAD. However, the results of the rank-ordered activity levels (supplementary analyses 3) of the amygdala do not indicate stress-related differences between the control and SAD groups . The cortical emotion regulation regions showed a trend towards relatively stronger activation in the control group during speech anticipation, perhaps this also points at increased cortical regulation. Our findings suggest that amygdala connectivity specifically differentiates the controls from the SAD patients. While the role of the amygdala is well established in ambiguity detection of unpredictable or salient stimuli (Davis & Whalen 2001; Whalen 2007), the role of the amygdala in prolonged social stress states is currently debated, as decreases have also been reported (Pruessner *et al.* 2008; Wager *et al.* 2009). Amygdala *activity* and *connectivity* may have partially different functions under certain circumstances. It is conceivable that amygdala activity (in its role of vigilance or ambiguity detector) is most strongly increased during for example face processing, and stress might increase reactivity to subsequent facial stimuli (van Marle *et al.* 2009; Oei *et al.* 2012). However, in its link to prolonged states of social stress itself, the role of the amygdala may be better understood as integrating cortical responses on the one hand, and subcortical responses (related to ANS and HPA activation) on the other, without necessary becoming more “active or inactive”.

There is an increasing interest in dynamic changes in connectivity within a measurement period (Chang & Glover 2009; Cribben *et al.* 2012), which is also a relevant factor to adequately capture the stress response. Qualitatively, the time-varying changes of cortical-amygdala connectivity during speech anticipation hint at the possibility of stronger earlier differences between SAD and controls for the right amygdala (see Figure S2). It has to be noted, however, that these interpretations are speculative, and no formal statistical significance between the two groups was observed. It is of interest that a recent paper, applying a paradigm similar to that applied in the current study, found time-varying effects in the occipital regions during early phase, and insula during recovery phase of social evaluative threat processing in SAD (Waugh *et al.* 2012).

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