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Task-dependent modulation of amygdala connectivity in social anxiety disorder

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ABSTRACT

Increased amygdala activation is consistently found in patients suffering from social anxiety disorder (SAD), a psychiatric condition characterized by an intense fear of social situations and scrutiny. Disruptions in the amygdalar-frontal network in SAD may explain the inability of frontal regions to appropriately down-regulate amygdalar hyper-activation.

In this study, we measured 15 SAD patients and 15 healthy controls during an affective counting Stroop task with emotional faces to assess the interaction of affective stimuli with a cognitive task in SAD, as well as to investigate the causal interactions between the amygdala and the medial orbitofrontal cortex (OFC) using dynamic causal modeling (DCM).

Here we show for the first time that differences in OFC-amygdala effective connectivity between SAD patients and healthy controls are influenced by cognitive load during task processing. In SAD patients relative to controls dysfunctional amygdala regulation was observed during passive viewing of harsh faces This could be linked to ongoing self-initiated cognitive processes (such as rumination and anticipation of negative events) that hinder successful amygdala regulation. However, between-group differences diminished during cognitive processing, suggesting that attentional load interfered with emotional processing in both patients and controls.

1. Introduction

Social anxiety disorder (SAD) is a psychiatric condition in which people suffer from intense dread of social situations and of other people's scrutiny. Studies focusing on its neural correlates have confirmed the central role of the amygdala (Davis and Whalen, 2001; Phelps and LeDoux, 2005), which is hyperactive in SAD patients compared to healthy controls (HC) during the perception of emotional facial expressions (Ball et al., 2012; Fitzgerald et al., 2006; Klumpp et al., 2012; Phan et al., 2006; Stein et al., 2002). Other studies have observed similar results using different types of stimuli that can be considered to be anxiety-inducing for SAD patients, such as reading negative comments referring to oneself (Blair et al., 2011), perception of social situations illustrated in images (Nakao et al., 2011), speech anticipation (Tillfors et al., 2001), direct eye gaze (Schneier et al., 2011, 2009), and situations involving uncertainty (Krain et al., 2008).

In addition to amygdalar hyperactivity, SAD has also been associated with aberrant activations of regions within the prefrontal cortex implicated in voluntary and automatic emotion regulation during anxiety-inducing stimuli (Hariri et al., 2003; Labuschagne et al., 2012; McClure et al., 2007; Monk et al., 2008; Phillips et al., 2008; Sladky et al., 2012). Disruptions of the amygdalar-prefrontal network in SAD have also been confirmed by resting-state functional connectivity studies (Hahn et al., 2011; Liao et al., 2010; Qiu et al., 2011).

While there is general agreement that SAD is characterized by dysfunctional amygdalar-prefrontal circuitry (for a review, see Bishop, 2007), little is known about the underlying SAD-specific temporal and causal dependencies within the network. Recently, we provided evidence for the inability of the medial orbitofrontal cortex (OFC) to down-regulate amygdalar hyperactivity in SAD patients relative to controls during exposure to emotional faces (Sladky et al., 2013b). Here, we aim to expand our previous findings by investigating the interaction between affective stimuli and a cognitive task in the clinical context of SAD. Based on previous work that has demonstrated the

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importance of attentional load and the availability of perceptual resources on emotion processing and regulation (Blair et al., 2007; Pessoa et al., 2005; Silvert et al., 2007; Schultz and Heimberg, 2008), we aim to investigate how simultaneous processing of a cognitive task during emotional face perception affects the effective connectivity between OFC and amygdala. For this purpose, the affective counting Stroop task proposed by Blair et al. (Blair et al., 2007) was used, in which emotional faces served as distractors during a goal-directed processing task with a varying attentional load.

Specifically, we used dynamic causal modeling (Friston et al., 2003) to assess the effective connectivity between the amygdala and OFC, which has been implicated as an important neuronal regulator (Phillips et al., 2008; Ray and Zald, 2012). During passive viewing we hypothesize that OFC would successfully inhibit the amygdalar activation during emotional processing in healthy controls, which down-regulation would be dysfunctional in patients. If cognitive load interferes with emotional processing, we expect to observe increased amygdalar activation in SAD, relative to HC, in the absence of cognitive demand, which should become attenuated during the cognitive task with increasing attentional load. On the other hand, if emotional processing of harsh distractors interferes with cognitive processing in SAD, we expect to see longer reaction times during the cognitive task in patients compared to controls, as suggested by previous findings (Blair et al., 2007).

2. Methods and materials

2.1. Study population

Fifteen SAD patients (7 males and 8 females, mean age ± SD: 26.6 ± 8.6 years) and 15 matched healthy control (HC) participants (8 males and 7 females, mean age ± SD: 25.4 ± 3.4 years) took part in the study. All participants provided written informed consent prior to the study and were financially reimbursed for their participation. The study was approved by the institutional advisory board of the Medical University of Vienna in accordance with the Declaration of Helsinki and national laws.

Both SAD patients and HC underwent clinical assessment by the psychiatrists of the Department of Psychiatry and Psychotherapy of the General Hospital in Vienna. None of the participants had any history of neurological or psychiatric disorders, with the exception of SAD in the patient group. Additional exclusion criteria included pregnancy, current or prior history of substance abuse, or any psychotropic medication within the last three months. On the day of the experiment, a compulsory drug screening was conducted using ToxiQUICK PAN-10 test panels (ACON Laboratories, San Diego, USA), which were negative for all participants.

All participants were tested with the German version of the Structured Clinical Interview for DSM-IV (SCID) (Eysenck, 1997). Additionally, they completed the State-Trait Anxiety Inventory, State (STAI-S) and Trait (STAI-T) versions (Spielberger et al., 1983), as well as the Hamilton Anxiety Rating Scale (HAM-A; Hamilton, 1959) and the Liebowitz Social Anxiety Scale (LSAS; Liebowitz, 1987). Based on the psychometric scores, SAD patients reported greater levels of social anxiety on the LSAS, HAM-A, and STAI (Table 1).

2.2. MRI Acquisition parameters and fMRI paradigm

Participants were scanned in a 3 T TIM TRIO MR scanner (Siemens Medical, Erlangen, Germany) using a high-sensitivity 32-channel head coil. 485 whole-brain volumes oriented along the AC–PC line with a matrix size of 128×128×20 voxels were acquired at a repetition time of TR=1.8 s, using a GE single-shot echo planar imaging (EPI) sequence (TE=40 ms, FOV=190×190 mm^2, voxel size=1.5×1.5×3 mm^3, inter-slice gap=2.1 mm, and bandwidth=1446 Hz/px). The purpose of the high spatial resolution was to minimize MRI signal losses in the ventral brain regions due to the local field inhomogeneity leading to intra-voxel dephasing effects (Robinson et al., 2004).

Table 1.
Psychometric data. All subjects were evaluated using Liebowitz Social Anxiety Scale (LSAS), State-Trait Anxiety Inventory (STAI-S/T), and Hamilton Anxiety Rating Scale (HAM-A). Table shows mean ± SD, two-tailed two-sample *t*-test used for group comparison.

Group	HC	SAD	*t*-test
Gender	7 f/8 m	8 f/7 m	
Age [years]	25.4 ± 3.4	26.6 ± 8.6	t_{28}=0.50, $p > 0.6$
LSAS	5.3 ± 7.3	75.6 ± 22.7	t_{28}=11.42, $p < 0.001$
HAM-A	0.5 ± 0.6	16.9 ± 5.0	t_{28}=12.61, $p < 0.001$
STAI-S	25.6 ± 3.3	42.1 ± 12.8	t_{28}=4.83, $p < 0.001$
STAI-T	27.0 ± 4.8	52.2 ± 11.2	t_{28}=8.01, $p < 0.001$

The Affective counting Stroop task (Blair et al., 2007) was adapted for the current study. In this task, participants were sequentially presented with two numerical displays in the form of a 3-by-3 matrix consisting of numbers and asterisks. Between the presentations of the numerical displays, we displayed distractors in the form of harsh faces (disgusted or angry) or scrambled, unrecognizable neutral faces, which were obtained from the NimStim facial stimulus set (Tottenham et al., 2009). An example of the presentation stimuli can be seen in Fig. 1. Participants had to decide whether the first or the second matrix displayed contained the greater quantity of digits, irrespective of the value of the displayed numbers. The task contained three different Stroop conditions: congruent (the number of digits corresponded to the value), incongruent (the number of digits differed from the value), and a passive viewing condition, in which asterisks were shown instead of numbers and no response from the participant was required. The conditions were presented in a randomized order. A crosshair was shown with jittered presentation duration (3400–7700 ms, uniform distribution) between the task events.

2.3. Preprocessing and general linear model (GLM) analysis of fMRI data

Data were preprocessed and analyzed using SPM12b (FIL Methods Group, Welcome Trust Center for Neuroimaging, University College London, http://www.fil.ion.ucl.ac.uk). We performed slice-timing correction (Sladky et al., 2011), realignment to compensate for movement, normalization to standard MNI space using an additionally acquired T_1-weighted MPR anatomical scan for each participant, and spatial smoothing (8 mm FWHM) to reduce inter-subject variability and increase signal-to-noise ratios.

Single-subject GLM analysis included regressors for Stroop task complexity (passive viewing, congruent and incongruent) and distractor valence (harsh faces, neutral) using boxcar functions aligned to stimulus presentation onsets, convolved with SPM's canonical HRF, and the six realignment parameter vectors taken from the realignment procedure. Thus, a separate block of trials was modeled for each of the following conditions: congruent harsh, congruent neutral, incongruent harsh, incongruent neutral, passive viewing harsh and passive viewing neutral. A second-level group analysis of the harsh-neutral contrast (irrespective of the cognitive load) was performed using one-sample *t*-tests across all participants to localize the bilateral amygdalae and OFC, comprising the emotion processing circuitry (Sabatinelli et al., 2011). Significance threshold for the statistical parametric map was set to $p < 0.001$ (uncorrected) with a minimum cluster extent of k=10 voxels (Lieberman and Cunningham, 2009). To verify the validity of this approach in the context of our study, we used an independent method to perform a cluster-level correction. An anatomically defined mask was created in SPM's Anatomy toolbox (Eickhoff et al., 2007, 2006, 2005), including our a priori regions of interests (k=940 vx). The modified version of AFNI's 3dClustSim, was used for statistical thresh-

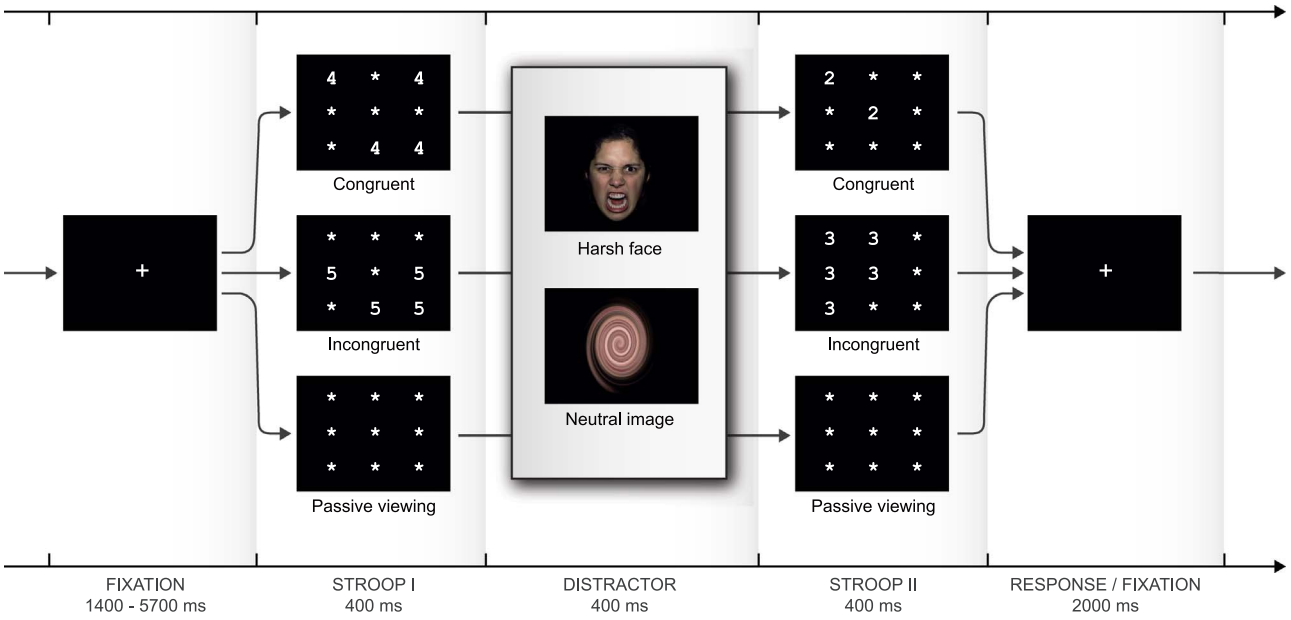

Fig. 1. Affective counting Stroop task. Participants performed a counting Stroop task that included emotional faces as affective distractors, comprising a randomized sequence of 40 congruent trials (value=number of digits), 40 incongruent trials (value≠number of digits), and 40 trials of passive viewing (no action required). The affective distractors were a balanced number of harsh-looking faces (disgusted or angry) or scrambled content-less neutral images.

olding. It performs a Monte Carlo simulation of the cluster size threshold for an alpha level of 0.01 and height threshold of 0.001, which resulted in k=7.3 voxels. As a consequence, we used the stricter threshold of k=10 voxels.

For DCM and ROI-based analyses, whitened and detrended time courses of the OFC and both amygdalae were extracted for each participant using SPM's volume of interest (VOI) extraction batch script (individual activation maxima within a search radius of r=10 mm from the second-level analysis used as centers, single-subject significance threshold $p < 0.05$, first eigenvariate used as summary statistic). Peak voxels for left amygdala: $x/y/z$=−20/−12/−10 mm [MNI], right amygdala: $x/y/z$=20/−10/−12 mm [MNI], and OFC: $x/y/z$=4/48/−18 mm [MNI].

For the subsequent ROI analyses, a second GLM was defined to assess the interaction between Stroop conditions and distractor valences on the amygdala using 6 regressors (3 Stroop conditions×2 distractors).

2.4. Dynamic causal modeling

Dynamic causal modeling (DCM) was applied for the investigation of effective connectivity between brain regions (Friston et al., 2003). DCM consists of a neuronal model that is related to empirically observed BOLD changes using a biophysical forward model (Balloon model Buxton et al., 1998). The neural state equation of DCM is given by:

$$\frac{dz}{dt} = (A + \sum u_j B^j) z + Cu$$

having vector $\boldsymbol{z}$ representing the time series of the neural behavior, vector $\boldsymbol{u}$ containing the time course(s) (1, ...,j, ..., n) of the external perturbation (i.e., the experimental paradigm), the internal steady state connectivity parameters $\boldsymbol{A}$, modulatory effects on these connections by stimulus u_j given by $\boldsymbol{B}$, and the direct influence of a stimulus modeled by $\boldsymbol{C}$. Therefore, the DCM equation describes a linear combination of internal and external influences (i.e., endogenous connectivity $\boldsymbol{A}$, modulation $\boldsymbol{B}$, and inputs $\boldsymbol{C}$) in order to explain neural activity as it is observed by functional MRI and other neuroimaging methods (e.g., DCM for EEG and MEG).

Motivated by our hypotheses and previous studies (Hahn et al., 2011; Sladky et al., 2013b), we investigated effective connectivity between OFC and amygdala using a separate model space for each hemisphere. Harsh and neutral distractors were used as driving inputs ($\boldsymbol{C}$) for the amygdala and all possible combinations of the three Stroop conditions were used as modulators ($\boldsymbol{B}$) on the forward and backward connections between OFC and amygdala ($2^{3\times2}$=64 models for each hemisphere).

Then, Bayesian Model Averaging (BMA) (Penny et al., 2010) was applied to infer on the posterior distribution of all parameter estimates within our predefined model space and subject population, weighted by the respective posterior probability of a model m for a given subject n. BMA thus accounts for uncertainties about the model structure and, at the same time, allows for inference on the distribution of the posterior parameter estimates (Stephan et al., 2010). We used classical frequentist inference (i.e., t-test) on the resulting connectivity parameter estimates and their modulations, applying Bonferroni correction to account for multiple comparisons ($p < 0.05$).

3. Results

3.1. Behavioral data

Reaction times (RTs) and accuracy in performance were compared between the two experimental groups, using the IBM SPSS Statistics software, Version 20.0. Mean RTs were computed for each participant and the effects of distractor valence and Stroop condition on RTs were examined using a 2 (distractor: negative, neutral) by 2 (task: congruent, incongruent) repeated measures ANOVA. There was neither a significant main effect of task, nor a significant main effect of emotion, nor an interaction within and between groups ($p > 0.1$). Note that the passive viewing condition had no observable behavioral outcome (Table 2). In summary, our findings do not provide any evidence for a modulatory role of emotional distractors on cognitive processing at the behavioral level.

3.2. Statistical parametric mapping

The contrast of harsh faces versus neutral distractors, combined for all cognitive tasks, across all participants (patients and controls) revealed significant task-specific activations in bilateral amygdala,

Table 2.
Behavioral data. Group averages (± SD) of response times (RTs) and task accuracy in combination with neutral and harsh distractors. Using repeated measures ANOVA, we neither observed a significant main effect of task, nor a significant main effect of emotion, nor an interaction within and between groups. Note that the passive viewing condition had no observable behavioral outcome.

Valence	Task	Group	RTs (± SD) [ms]	Accuracy (± SD) [%]
Neutral	Congruent	HC	756 (± 249)	95 (± 4)
		SAD	834 (± 290)	97 (± 2)
	Incongruent	HC	818 (± 332)	94 (± 7)
		SAD	844 (± 270)	96 (± 3)
Harsh	Congruent	HC	730 (± 217)	94 (± 6)
		SAD	816 (± 328)	96 (± 4)
	Incongruent	HC	745 (± 227)	94 (± 7)
		SAD	829 (± 297)	98 (± 4)

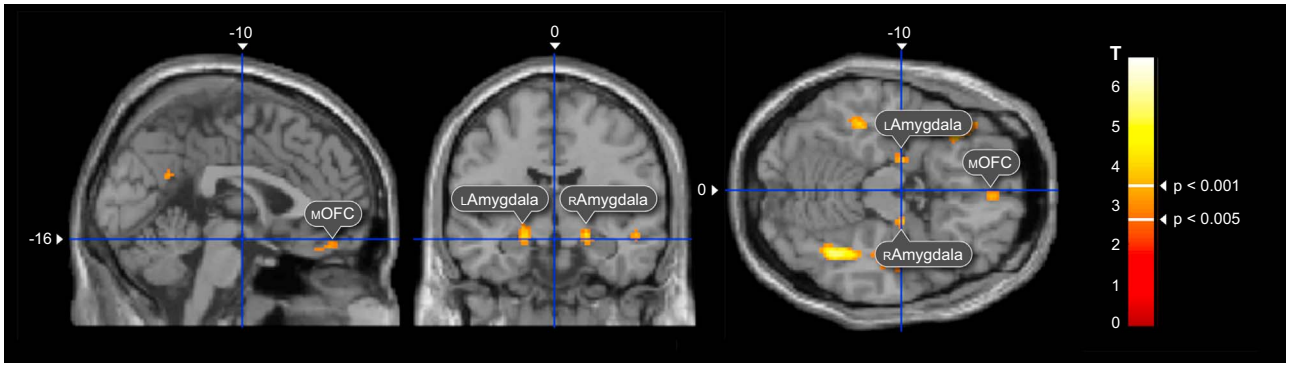

Fig. 2. SPM *t*-statistic for harsh-neutral distractor conditions (SAD patients and healthy controls combined). Significant activations in bilateral amygdala, fusiform gyrus and other temporal lobe regions and the prefrontal cortex including the medial OFC. Whole-brain significance threshold $p < 0.001$ (uncorrected), k=10vx minimum cluster size; $p < 0.005$ used in figure for display purposes. Crosshair position $x/y/z$=0/−10/−16 mm [MNI]. Peak voxels for left amygdala: $x/y/z$=-20/−12/−10 mm [MNI], right amygdala: $x/y/z$=20/−10/−12 mm [MNI], and OFC: $x/y/z$=4/48/−18 mm [MNI].

fusiform gyrus and other temporal lobe regions and the prefrontal cortex including the medial OFC (Fig. 2, Supplement Table S1, cluster-corrected using AFNI's 3dClustSim). Based on this functional localization and our previous studies (Sladky et al., 2013a, 2013b, 2012), a subsequent ROI analysis was conducted to investigate the influence of the individual Stroop conditions on amygdalar activation.

Compared to HC, SAD patients showed significant bilateral amygdalar hyperactivity during passive viewing, with the strongest effect observed for the right amygdala. During the cognitive task conditions (congruent, incongruent), between-group differences were evident only in the left amygdala and only during the congruent condition (Fig. 3). The amygdala was equally activated bilaterally in both groups during the incongruent condition.

3.3. *Effective connectivity results*

Endogenous (i.e. task-independent) connectivity provides insights into the neuronal connectivity between brain regions that is constant across all experimental conditions. In our DCM results, all endogenous connections were significantly different from zero, with top-down connections from OFC to both amygdalae being inhibitory in nature, while bottom-up connections from both amygdalae to OFC – excitatory (Figs. 4 and 5; Table S2).

In terms of endogenous connectivity, SAD patients and controls differed only in the extent to which bottom-up endogenous connections were excitatory (e.g. more positive connections from the left amygdala to OFC in SAD, relative to HC, and a reversed trend from the right amygdala to OFC).

The modulatory, or context-dependent, effects show how task perturbations modify the connectivity between brain regions. For both hemispheres, the DCM analysis showed that task-dependent modulation during passive viewing was negative indicating that OFC inhibition of the amygdala was further amplified, but only in healthy controls. In SAD patients, however, task-dependent modulation during passive viewing was positive, thereby changing the initially inhibitory feedback from OFC to amygdala to an excitatory connection, i.e., leading to increased amygdala activity (Fig. 5, Table S2).

In general, higher cognitive demand by the Stroop conditions positively modulated OFC to amygdala connectivity, except in the right hemisphere of SAD patients where modulation was not significant. The most pronounced difference in SAD compared to controls in the amygdala to OFC direction was also a significant overall right-lateralized reduction in endogenous connectivity (Fig. 5).

4. Discussion

In the present study, we used an emotional Stroop task paradigm to investigate the interaction between a cognitive task with varying attentional load and the perception of emotional distractors (i.e., harsh faces) in SAD patients compared to healthy controls.

Based on our behavioral data, we found no evidence that emotional distractors interfered with cognitive processing in SAD patients, which should have led to prolonged reaction times in patients relative to controls. This is in contrast to previous findings (Blair et al., 2007) that showed a disruption in goal-directed processing caused by the presence of emotional distractors. However, this discrepancy in findings could have been caused by the difference in stimuli cues presented in the two studies. Contrary to our expectations, negative images from the International Affective Picture System, which were used by Blair and colleagues (2007), might have been more aversive and distracting for SAD patients than the emotional faces used in our study.

Next, we aimed to investigate whether attentional load interfered with emotional processing in SAD patients relative to controls. An initial analysis on the differences in brain activity patterns for harsh faces against neutral distractors clearly showed a network that responds to emotional faces (Sabatinelli et al., 2011), most importantly

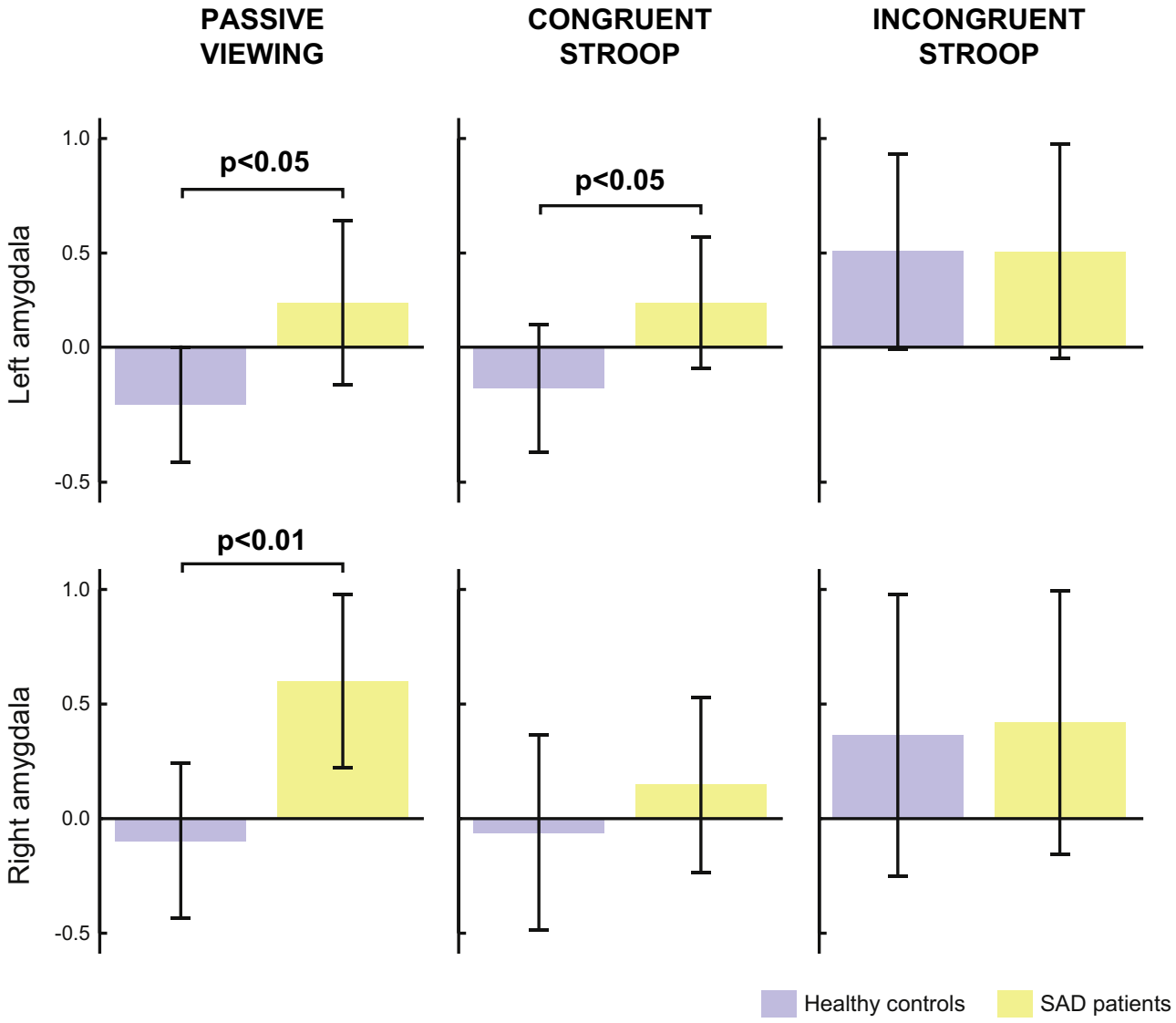

Fig. 3. ROI analysis in both amygdalae for task-dependent interactions for harsh-neutral contrast. Compared to HC, amygdalar hyperactivation in SAD patients was found to be associated with reduced cognitive load of the congruent Stroop and passive viewing condition. The highest amygdala activation was found in both groups for the incongruent Stroop condition. Second-level fMRI results were used for ROI definition (Figure 2). Percent signal change and 95% confidence intervals, all p-values Bonferroni-corrected. Blue: healthy controls, yellow: SAD patients. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

the amygdala and the OFC, which then served as a basis for the ROI definition and the subsequent DCM analysis.

To examine the effects of the cognitive tasks on emotional processing, we first conducted a ROI-based ANOVA analysis to assess differences in amygdalar activation modulated by cognitive load (i.e., passive viewing, congruent and incongruent conditions).

Our results showed that in the absence of a cognitive task (i.e., during passive viewing) SAD patients relative to controls exhibit higher activation in the amygdala. This effect was found bilaterally, but was stronger for the right amygdala. For the congruent (medium cognitive load) condition a trend towards higher activation in SAD patients was found for both amygdalae, however, this effect was not significant after applying Bonferroni correction. No group differences were observed for both hemispheres during the (high cognitive load) incongruent condition. These findings clearly demonstrate that attentional load does have an impact on amygdalar activation levels in SAD.

The consequent DCM analysis aimed to assess the effective connectivity between the amygdalae and OFC. We expected that, given the down-regulating function of the prefrontal cortex on the amygdala, the perception of harsh faces in SAD would be influenced by the increasing demand for cognitive resources during higher attentional load. During emotion regulation, it has been proposed that the OFC plays a central role for integration and modulation of neural activation in the amygdala and other regions in order to monitor and control emotional responses (Rule et al., 2002). Regarding the functional network between OFC and amygdala, previous research suggests that SAD patients exhibit decreased connectivity, i.e., (1) structural connectivity (Baur et al., 2013), (2) resting-state functional connectivity (Hahn et al., 2011; Liao et al., 2010), and (3) effective connectivity when confronted with emotional faces (Sladky et al., 2013b). Our results corroborate these studies in demonstrating a lack of amygdala inhibition in SAD patients when confronted with harsh faces in the passive viewing condition, which was more pronounced in the right amygdala. In addition, our results also show that these group differences diminish with higher cognitive demand, i.e. in the congruent and incongruent conditions, respectively. It seems that when a task requires more cognitive resources, down-regulation from OFC to amygdala becomes insufficient.

The differential effects observed between the left and right amygdala also suggest that lateralization effects in amygdalar activation levels might indeed be important factors in the physiology and pathophysiology of emotional face processing. While a significant body of research has consistently reported amygdala activation during

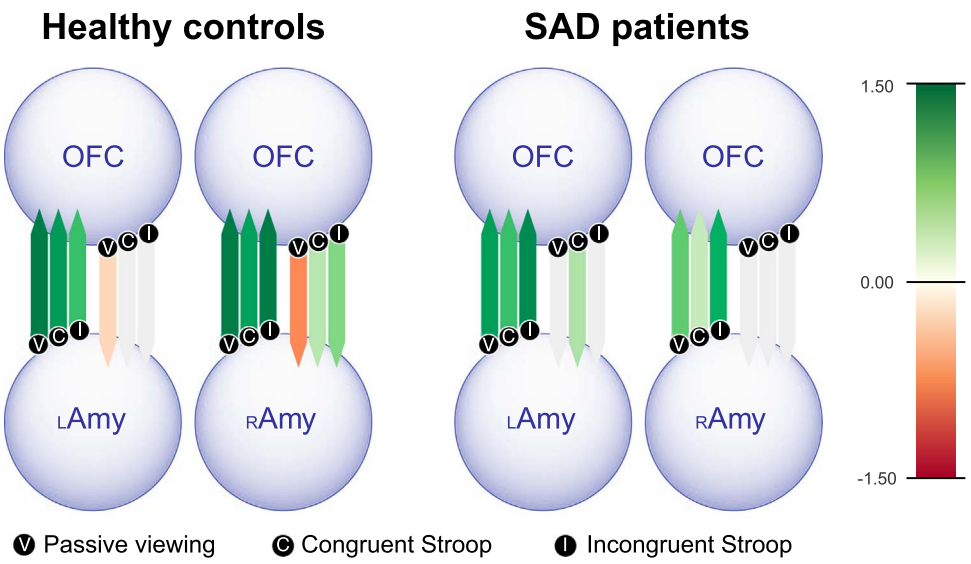

Fig. 4. Dynamic causal modeling (BMA) group results for healthy controls and SAD patients. The most important finding was that significant down-regulation of the amygdala during VIEWING was only observed in healthy controls and not in SAD patients. Harsh and neutral distractors were used as driving inputs (***C***) for the amygdala. Connectivity parameter estimates [s^{-1}] are color-coded (green: positive, red: negative, grey: not significant [$p < 0.05$, Bonferroni corrected]). Arrows indicate task-dependent effective connectivity (***A*** ***+B****) for the viewing, congruent, and incongruent Stroop conditions. Two independent model spaces were used for each hemisphere. (* A+B indicates that B matrix posterior parameters are over and above the A-matrix posterior parameters). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

emotion processing, it is inconclusive whether there is a clear-cut hemisphere-specific difference between the right and left amygdala (see Baas et al. (2004) for a systematic review of previous studies). One of the proposed hypotheses is that the right amygdala is engaged in fast analysis of emotional stimuli, while the left amygdala is involved in more detailed feature extraction (Markowitsch, 1998; Phelps et al., 2001). A similar hypothesis was proposed by Glascher and Adolphs (2003), who suggested that the right amygdala is first automatically activated by emotional stimuli, while the left amygdala is engaged in cognitive perceptual emotional information processing. In the light of this framework and our findings, it can be argued that, at least in the right amygdala, SAD patients processed the negative stimuli in a bottom-up fashion, irrespective of the cognitive task. Thus, the cognitive task did not modulate the endogenous connectivity between OFC and right amygdala.

With regard to the laterality effects observed in the amygdalae, it would be of interest whether there is differential hemispheric effect of the OFC, as well. However, without the presence of a clearly lateralized OFC activation, we cannot address this issue in the present study. The peak activated by our task was located in the right hemisphere, close to the midline, and was used as the only OFC peak for the DCM analysis. While there is limited evidence for the differential role of the two hemispheres, recent studies have emphasized the importance of cytoarchitectonic and functional heterogeneity of the OFC (Bludau et al., 2014; Henssen et al., 2016), as well as the division between lateral and medial OFC, the latter more involved in emotional regulation. Based on the SPM's Anatomy toolbox, the OFC peak in our analysis is located between the medial frontopolar cortex (Fp2; Bludau et al., 2014) and Fo2 sub-region of the OFC (Henssen et al., 2016), with higher similarity between these areas (see Figure 13 in Henssen et al.,

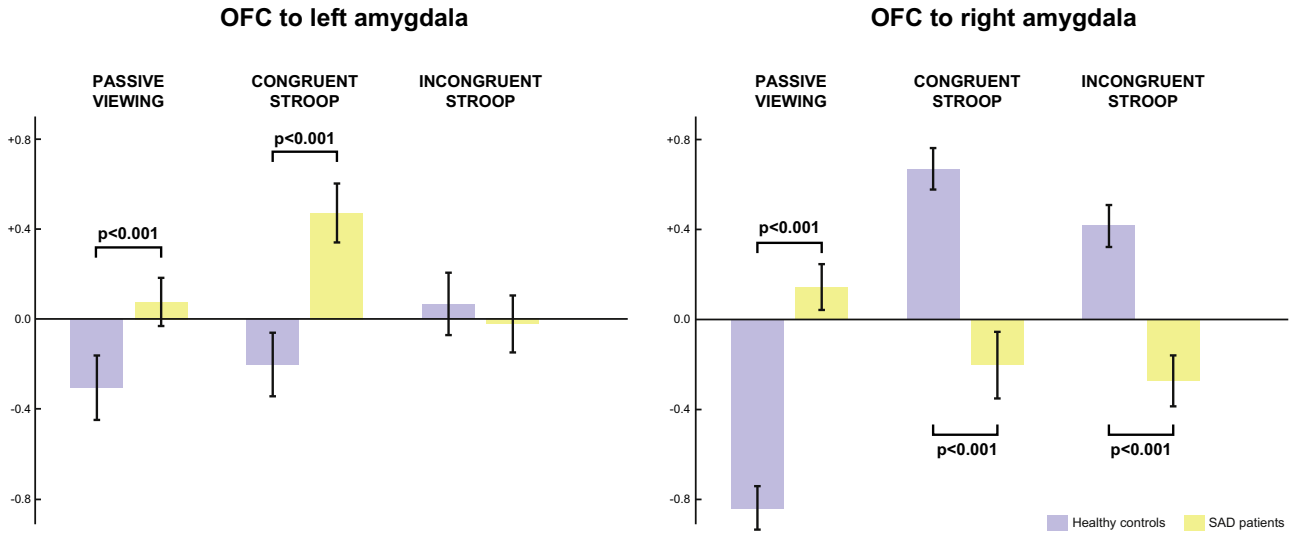

Fig. 5. Task-dependent effective connectivity (A+B*) between orbitofrontal cortex and amygdala in the right and left hemisphere. The passive viewing condition negatively modulated the OFC to amygdalar connection in healthy controls, which was not the case in SAD patients, where a positive modulation was found. BMA group results ± standard deviation [a.u.]; *t*-test used for statistical inference ($p < 0.05$, Bonferroni-corrected); blue: healthy controls, yellow: SAD patients. (* A+B indicates that B matrix posterior parameters are over and above the A-matrix posterior parameters). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

2016) and both responsible for emotion regulation. In summary, we show that regulation of the amygdala by the OFC is modulated by cognitive load. SAD patients showed increased activation in the amygdala during low cognitive load (congruent Stroop and passive viewing conditions), while there was no difference in the high cognitive load condition (incongruent Stroop condition). Our connectivity analysis showed significant down-regulation of the amygdala by the OFC in healthy subjects during the passive viewing condition. This indicates that healthy subjects, when not engaged in demanding tasks are able to efficiently down-regulate amygdalar activation. The fact that this was not observed in SAD patients suggests that there are neurobiological deficits in emotion regulation in these patients. Importantly, group differences vanish with high cognitive load, suggesting that attentional demand interferes with emotional processing in both groups. This could explain why the same pattern of absent amygdala regulation during cognitive load (congruent and incongruent Stroop task performance) that we found in healthy subjects was also observed in SAD patients when they performed a cognitive task. It can be speculated that only when cognitive resources are available (such as during passive viewing) do patients allocate them to (negatively-biased) self-referential thought processes, such as rumination and anticipation of negative events, leading to amygdalar hyperactivity.

One limitation of this study is that the sample size was limited to 15 SAD patients and 15 HC and therefore did not allow to distinguish subtypes of social anxiety disorder or to find robust associations between activation and connectivity parameter estimates and clinical scores. Thus, future studies with larger sample sizes should ideally address these issues and aim at replicating the results presented here. Nevertheless, differences in effective connectivity shown herein had sufficient statistical power as demonstrated by their significance at Bonferroni-corrected level. In our case, the small sample size speaks for the non-triviality of the observed effects (Friston, 2012, 2013). Significance thresholds to report fMRI SPM results were defined using AFNI's 3dClustSim based on Monte Carlo simulation of the cluster size threshold and a pre-defined anatomical mask, although a more conservative correction for multiple comparisons would have been desirable in order to decrease false positive rates. However, the second-level model was used to serve the DCM definition. Our DCM model space was motivated by the models used in previous work (Sladky et al., 2015, 2012). In addition, using a bootstrapping test, DCM connectivity parameter estimates between amygdala and OFC during face processing were shown to be highly robust for group sizes of more than 8 individuals.

The most important limitation of this study is that it does not investigate any psychopharmacological or psychotherapeutic treatment effects. Here, we studied a population of unmedicated SAD patients, who were scanned within a few days after their initial admission and before the onset of their facultative therapy plan. However, our previous placebo-controlled study on the effects of SSRIs during the processing of emotional faces in healthy subjects (Sladky et al., 2015; Windischberger et al., 2010) showed that down-regulation of the amygdala by the OFC is increased by (S)-citalopram (escitalopram). Phan et al. (2013) observed a reduction of amygdala hyperactivation in SAD patients after treatment with sertaline along with increased activation of the OFC when presented with fearful and angry faces. Given that the paradigm used in the present study provides a nuanced method to study the interaction of amygdala and OFC in the context of varying levels of cognitive load, it might be suitable to further investigate and differentiate medication effects in this important regulation network.

Contributors

RS, GSK, CK, RL, and CW designed and implemented the experiment. RS performed the fMRI measurements. GK, CK, and RL provided clinical support. LM, RS, MW, NG conducted data processing and analyses. RS created the figures. LM, RS, and CW wrote the initial manuscript. All authors revised and reviewed the manuscript.

Conflict of interest

R. Lanzenberger received travel grants and/or conference speaker honoraria from AstraZeneca, Lundbeck A/S, Dr. Willmar Schwabe GmbH, AOP Orphan Pharmaceuticals AG, Janssen-Cilag Pharma GmbH, and Roche Austria GmbH. M. G.S. Kranz received travel grants from Roche and Pfizer. C. Kraus received travel grants from Roche Austria GmbH and AOP Orphan Pharmaceuticals AG. L. Minkova, R. Sladky, M. Woletz, N. Geissberger, and C. Windischberger reported no financial interests or potential conflict of interest.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.pscychresns.2016.12.016.

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