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Research report

Separating depressive comorbidity from panic disorder: A combined functional magnetic resonance imaging and machine learning approach

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ABSTRACT

Background: Depression is frequent in panic disorder (PD); yet, little is known about its influence on the neural substrates of PD. Difficulties in fear inhibition during safety signal processing have been reported as a pathophysiological feature of PD that is attenuated by depression. We investigated the impact of comorbid depression in PD with agoraphobia (AG) on the neural correlates of fear conditioning and the potential of machine learning to predict comorbidity status on the individual patient level based on neural characteristics.

Methods: Fifty-nine PD/AG patients including 26 (44%) with a comorbid depressive disorder (PD/AG+DEP) underwent functional magnetic resonance imaging (fMRI). Comorbidity status was predicted using a random undersampling tree ensemble in a leave-one-out cross-validation framework.

Results: PD/AG−DEP patients showed altered neural activation during safety signal processing, while +DEP patients exhibited generally decreased dorsolateral prefrontal and insular activation. Comorbidity status was correctly predicted in 79% of patients (sensitivity: 73%; specificity: 85%) based on brain activation during fear conditioning (corrected for potential confounders: accuracy: 73%; sensitivity: 77%; specificity: 70%).

Limitations: No primary depressed patients were available; only medication-free patients were included. Major depression and dysthymia were collapsed (power considerations).

Conclusions: Neurofunctional activation during safety signal processing differed between patients with or without comorbid depression, a finding which may explain heterogeneous results across previous studies. These findings demonstrate the relevance of comorbidity when investigating neurofunctional substrates of anxiety disorders. Predicting individual comorbidity status may translate neurofunctional data into clinically relevant information which might aid in planning individualized treatment. The study was registered with the ISRCTN: ISRCTN80046034.

1. Introduction

Depression is the most frequent comorbid condition in panic disorder with agoraphobia (PD/AG), affecting nearly every second patient (Emmrich et al., 2012; Goodwin et al., 2005; Kessler et al., 2006; Roy-Byrne et al., 2000). Comorbid depression is associated with more severe panic symptomatology (Emmrich et al., 2012; Roy-Byrne et al., 2000) and overall impairment (Roy-Byrne et al.,

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2000). Yet, our knowledge about shared and distinct pathophysiological mechanisms in PD/AG patients with or without comorbid depression is limited; research on the neural substrates of PD/AG may be biased if comorbid depression is present. Beyond this phenotype perspective on the group level, there is a critical need to translate neuroimaging findings into clinically useful information for the individual patient. Multivariate pattern recognition employing machine learning generates predictive information that can be used for single subject classification, thus offering new tools to translate neuroimaging findings into diagnostic value for the individual patient (Orru et al., 2012). Hence, we complemented our conventional group analysis on the effects of depressive comorbidity on the neural substrates of fear conditioning by predicting depressive comorbidity status for the individual patient within a machine-learning framework.

Fear conditioning is a basic learning process with fundamental relevance for the survival of an organism. During differential fear conditioning, contingencies are established by pairing an aversive unconditioned stimulus (US) with a previously neutral stimulus (conditioned stimulus; CS+). While the CS+ signals are the potential presence of a threat, a second neutral stimulus (CS−) that is never paired with the US becomes a signal for safety. The neural network of fear conditioning has been extensively studied in humans using functional magnetic resonance imaging (fMRI). Extending animal research focusing on the amygdala as a key region (LeDoux et al., 1988), further cortical and subcortical networks encompassing the thalamus, amygdala, hippocampus, insula, anterior cingulate cortex (ACC) and prefrontal/orbitofrontal cortex (PFC/OFC) have been shown to be activated during human fear conditioning (Sehlmeyer et al., 2009). Of note, this "threat network" has substantial overlap with structures that show abnormal activation in different anxiety disorders (Etkin and Wager, 2007).

Fear conditioning represents a central pathway for the development and maintenance of PD/AG (Bouton et al., 2001; Dresler et al., 2012; Kircher et al., 2013; Lueken et al., 2014), but the precise nature of fear learning deficits still remains under debate. Behavioral studies showed that PD may be characterized by excessive fear responding toward the safety signal (CS−) when compared to the threat signal (CS+) (Lissek et al., 2009). In line, using an instructed fear conditioning paradigm Tuescher et al. (2011) demonstrated increased neural activation in threat network structures such as the subgenual cingulate, ventral striatum and extended amygdala, as well as in the midbrain periaqueductal gray during the processing of safety cues compared to the threat condition. This response pattern was specific for PD when compared to posttraumatic stress disorder (PTSD) patients. However, the influence of comorbid depression on the reported brain activation pattern remains unresolved, since comorbid patients were not excluded and subgroup analyses were not feasible in this sample of eight patients. In a similar vein, we (Lueken et al., 2013) recently reported increased activation in a network related to threat (anterior cingulate cortex, hippocampus, and amygdala) during fear conditioning in response to the safety signal (CS−) compared to the threat signal (CS+) as a pre-treatment feature of non-response to CBT in a sample of medication-free PD/AG patients. However, depression comorbidity was allowed unless being clinically the primary diagnosis (Gloster et al., 2011).

While increased responding to stimuli that signal safety may underlie the onset and maintenance of anxiety disorders (Duits et al., 2015), recent evidence suggests that this pathomechanism is moderated by the presence of comorbid depression. The magnitude of fear reactions under safe conditions is a specific risk factor for the development of anxiety, but not depressive disorders (Craske et al., 2012). Specifically, startle potentiation under different threat conditions was diminished in PD when comorbid depression was present (Melzig et al., 2007). Similar findings on impaired fear inhibition toward safety signals as a feature of anxiety, but not depression have been reported in PTSD (Jovanovic et al., 2010). The influence of comorbid depression on the neural correlates of safety signal processing in PD/AG remains still unresolved as previous studies did not consistently report or control for comorbid depression, which can be mainly subjected to power restrictions in small-scale studies.

The aim of the present study therefore was twofold: using a comprehensive sample of 59PD/AG patients with a substantial proportion exhibiting a comorbid depressive disorder, we investigated whether the neural correlates of safety signal processing differed between PD/AG patients with and without comorbid depressive disorders (PD/AG+DEP; PD/AG-DEP). Second, translating these findings into clinically useful information, we tested the potential of machine learning to predict depressive comorbiditiy on an individual patient level based on neural characteristics. Following previous evidence from behavioral investigations (Craske et al., 2012; Duits et al., 2015; Melzig et al., 2007) we hypothesized that altered safety signal processing in brain areas subserving the detection of threat (e.g. amygdala, anterior cingulate cortex, and insula) should be most pronounced in patients without comorbid depression. Dimensional markers of panic symptomatology, but not depression, were expected to correlate with the magnitude of neurofunctional activation patterns during safety signal processing.

2. Methods

2.1. Subjects

As a part of the German research network "PANIC-NET", including a randomized controlled clinical trial on exposure-based CBT in PD/AG (Gloster et al., 2011), current results represent a secondary analysis supplementing the main fMRI publication (12). Eight German centers participated in the clinical trial (Aachen, Berlin-Adlershof, Berlin-Charité, Bremen, Dresden, Greifswald, Münster, Würzburg) treating 369 patients who met DSM-IV-TR criteria for PD/AG. At four centers (Aachen, Berlin-Charité, Dresden, Münster) an fMRI add-on study was conducted. From 369 patients enrolled in the clinical trial, 194 were recruited at fMRI centers, and of these 89 patients consented to participate in the present study. 60 Quality controlled baseline data sets were available. Details on the study protocol (including a CONSORT flowchart), in- and exclusion criteria and measures of fMRI data quality control are given elsewhere (Gloster et al., 2011; Kircher et al., 2013). One patient without complete diagnostic information on comorbidity patterns had to be excluded, leaving $n=59$ patients for the present analysis. Briefly, currently only (i.e. 4-week washout period) medication-free patients with a primary diagnosis of PD/AG according to DSM-IV-TR criteria as assessed by a standardized interview (Composite International Diagnostic Interview (CAPI-WHO-CIDI; DIAX-CIDI version; Wittchen and Pfister, 1997)) which was validated by clinical experts, a Hamilton Anxiety Scale Score (SIGH-A; Shear et al., 2001) ≥ 18, a Clinical Global Impressions Score (CGI; Guy, 1976) ≥ 4 and aged 18–65 years were included. Inability to comply with the study schedule, clinically significant suicidal intent, diagnostic criteria for any psychotic or bipolar disorder, borderline personality disorder, or current alcohol dependence, medical conditions explaining anxiety symptoms and MRI-related contraindications were followed by exclusion. Current comorbid diagnoses, including major depression, dysthymia and other anxiety disorders were allowed unless they were of primary clinical concern. As such, this sample can be considered both relatively severe and representative of patients

seen in clinical practice. For the present analysis, patients with PD/AG were grouped according to the presence of comorbid depression (DSM-IV-TR 12-month diagnosis of major depressive disorder and/or dysthymia). Twenty-six patients (44.1%) met criteria for a depressive disorder (PD/AG+DEP), while 33 (55.9%) patients presented without depression (PD/AG−DEP). The study had been conducted in compliance with the Declaration of Helsinki and had been approved by all ethics committees of the participating centers. Participating subjects provided written informed consent. Demographic and clinical characteristics between groups were tested using χ^2 and *t*-tests. A significance threshold of $P<0.05$ was applied. Analyses were carried out using SPSS, version 21.0 (IBM, Armonk, N.Y.).

2.2. Fear conditioning task

We applied a previously validated (Lueken et al., 2014; Reinhardt et al., 2010) differential fear conditioning task with colored geometric stimuli as CSs (presentation time: 2000 ms with a variable inter-trial interval (ITI) of 4.785–7.250 s) and an aversive auditory tone (white noise; 100 ms) as US. The task consisted of three phases: familiarization, with 16 trials of each CS, acquisition with 32 trials of each CS; and extinction, with 16 trials of each CS. In the acquisition, the US was paired pseudo-randomly with one of the CSs (CS+ paired trials; counterbalanced between subjects; partial reinforcement rate of 50%), resulting in equal proportions of CS+ paired and CS+ unpaired trials. To avoid confounding effects between CS+ and US processing, only CS+ unpaired trials were analyzed during acquisition. After each phase, subjective valence and arousal ratings using the self-assessment Manikin (SAM; Bradley and Lang, 1994) for both CSs were obtained using a five-point Likert scale (for valence: 1, "very unpleasant" to 5, "very pleasant" and for arousal: 1, "not arousing" to 5, "very arousing" $n=1$ subject missing due to technical failure). Behavioral ratings as indices for contingency knowledge have been reported elsewhere (Kircher et al., 2013); differences between comorbidity groups are additionally presented here. Task duration was 16 min 49 s. Stimuli were presented by MR-compatible LCD goggles or back-projection systems and standard headphones using Presentation 11 (Neurobehavioral Systems; www.neurobs.com).

2.3. fMRI data acquisition and analysis

As previously described (Kircher et al., 2013) images were acquired using 3-T Philips Achieva (Aachen, Münster), 3-T Siemens Trio (Dresden), and 3-T General Electric Healthcare (Berlin) scanners. 505 axial functional images (matrix=64×64; 30 slices interleaved; field of view=230; voxel size=3.6×3.6×3.8 mm; TE=30 ms; TR=2 s), covering the whole brain and positioned parallel to the intercommissural line (anterior commissure–posterior commissure) were recorded. The first five volumes were discarded to minimize T1 saturation effects. MR images were preprocessed using SPM5 (www.fil.ion.ucl.ac.uk) implemented in MATLAB, version 7.1 (MathWorks, Natick, Mass.), applying a high-pass filter (cutoff period, 128 s) to remove low-frequency fluctuations in the blood-oxygen-level-dependent (BOLD) signal. Following slice time correction, functional images were temporally and spatially aligned and normalized into a standard stereotactic space (Montreal Neurological Institute template: 2×2×2 mm). An iterative smoothness equalization procedure (Friedman et al., 2006) was performed with a 12-mm full width at half maximum Gaussian isotropic kernel (which is comparable to a kernel of 8 mm in a normal smoothing procedure). At first level, realignment parameters were added as regressors of no interest. We modeled the BOLD response for each event type (CS+ paired, CS+ unpaired, CS− and US) and phase (familiarization, acquisition, and extinction) convolved with the canonical hemodynamic response function used in SPM5 within the framework of the general linear model. Each phase was divided into an early and a late half to account for temporal aspects and habituation, resulting in 16 regressors (familiarization: early CS+, late CS+; early CS−, late CS−; US; acquisition: early CS−, late CS−, early CS presented with the US (CS+ paired); early CS+ without US (CS+ unpaired), late CS+ paired; late CS+ unpaired; US; extinction: early CS−, late CS−; early CS+, late CS+; behavioral assessment). In the present analysis, early and late phases were again averaged due to power considerations. Parameter estimates (beta values) and *t*-statistic images were calculated for each subject.

The group analysis was performed by entering contrast images into a flexible factorial analysis, in which subjects were treated as random variables. Regressors of interest from the first level (as stated above) were contrasted against the implicit baseline (fixation cross) and incorporated into the second level model. Thus, both CS+ and CS− were included to test the interaction effect of group and CS (separately for the acquisition and extinction phase). As in previous analyses (Kircher et al., 2013; Lueken et al., 2013, 2014), we included an fMRI center variable to account for scanner differences between sites. Since PD/AG+DEP patients were older and exhibited higher SIGH-A scores (Table 1), age and SIGH-A scores were entered as covariates of no interest into the model (in addition to gender and educational level, see Kircher et al. (2013), Lueken et al. (2013, 2014)). Separately for acquisition and extinction phases, *F*-contrasts were computed for the main effect of group (PD/AG+DEP vs. PD/AG−DEP) and the group × CS interaction, followed by post-hoc *t*-contrasts to specify the direction of the effect (acquisition: PD/AG−DEP > PD/AG+DEP, PD/AG+DEP > PD/AG−DEP, PD/AG−DEP > PD/AG+DEP (CS+ unpaired > CS−), PD/AG+DEP > PD/AG−DEP (CS+ unpaired > CS−); extinction: PD/AG–DEP > PD/AG+DEP, PD/AG+DEP > PD/AG−DEP, PD/AG−DEP > PD/AG+DEP (CS+ > CS−), PD/AG+DEP > PD/AG−DEP (CS+ > CS−)). Paralleling previous analyses, a Monte Carlo simulation of the brain volume was conducted to establish an appropriate voxel contiguity threshold (Slotnick et al., 2003). Assuming an individual voxel type I error at $P<0.005$, a cluster extent of 142 contiguous resampled voxels was indicated as sufficient to correct for multiple voxel comparisons at $P<0.05$. Since this approach could bias findings toward larger brain regions, a region-of-interest analysis of the amygdala was conducted using the Wake Forest University PickAtlas (Maldjian et al., 2003) ($P<0.05$, family-wise-error corrected with a minimum cluster size of 10 voxels). Beta values from significantly activated brain clusters were extracted and used for correlational analyses (Pearson's correlations) with panic- and depression-specific measures (Beck Depression Inventory (BDI II; Beck et al., 1996), Panic and Agoraphobia Scale (PAS; Bandelow, 1999) and Anxiety Sensitivity Index (ASI; Peterson and Reiss, 1992)) within subgroups and the entire patient group.

Ratings were analyzed using a three-factorial analysis of variance with the between-subjects factor group and the two within-subjects factors CS and time. Greenhouse–Geisser corrections were applied when appropriate. The statistical threshold was set at $P<0.05$.

2.4. Multivariate classification approach

Individual patient comorbidity prediction was performed using the Random Under Sampling Boost algorithm (Seiffert et al., 2010) as implemented in Matlab using default parameters with a minimum leaf size of 5 and a learning rate of 0.1 growing 500 trees on the whole-brain beta images separately for the two main task contrasts of interest (acquisition: CS+ unpaired > CS−; extinction: CS+ > CS− that have been used in previous analyses; see Hahn et al. (2015), Lueken et al. (2013)), as this algorithm is optimally suited to handle imbalanced class probabilities as present in this sample (Seiffert et al., 2010). Note that before classification, all data was transformed to a

Table 1
Demographic and clinical characteristics of the sample. Means (SD), except where noted.

	All patients ($n=59$)		PD/AG−DEP ($n=33$)		PD/AG+DEP ($n=26$)		χ^2 or t (df)	P
Demographic characteristics								
Female gender [n (%)]	40	(67.80)	21	(63.36)	19	(73.10)	0.594 (1)	0.441
Years of education [n (%)]								
8	4	(8.16)	2	(6.06)	2	(7.69)	0.064 (2)	0.969
10	30	(50.85)	17	(51.51)	13	(50.00)		
12–13	25	(42.37)	14	(42.42)	11	(42.31)		
Age (years)	36.66	(10.36)	34.10	(8.74)	39.92	(11.47)	−2.211 (57)	**0.031**
Clinical characteristics								
Comorbid major depression [n(%)]	–	–	–	–	19	(73.10)	–	–
Comorbid dysthymia [n (%)]	–	–	–	–	13	(50.00)	–	–
Age of onset depression/dysthymia (years)	–	–	–	–	29.92	(11.51)	–	–
Age of onset – PD/AG (years)[a]	27.16	(10.15)	24.88	(9.12)	30.35	(10.84)	−2.028 (53)	**0.048**
Number of comorbid psychiatric diagnoses within 12 months								
0	7	(11.86)	7	(21.21)	0	(0.00)	9.071 (3)	**0.028**
1–2	26	(44.07)	16	(48.48)	10	(38.46)		
3–4	18	(30.51)	7	(21.21)	11	(42.31)		
5+	8	(13.56)	3	(9.09)	5	(19.23)		
CGI	5.37	(0.67)	5.33	(0.54)	5.42	(0.81)	−0.510 (57)	0.612
SIGH-A total	24.42	(5.06)	23.27	(4.38)	25.88	(5.56)	−2.109 (57)	**0.048**
PAS total	27.29	(8.35)	25.61	(9.15)	29.42	(6.79)	−1.836 (57)	0.072
ASI total	31.41	(9.88)	32.48	(9.27)	30.04	(10.63)	0.943 (57)	0.350
BDI II total	17.66	(8.58)	14.91	(6.49)	21.15	(9.70)	−2.822 (42)	**0.007**
Neuropsychological characteristics								
Digit span forward	7.63	(1.95)	7.76	(1.94)	7.46	(1.98)	0.577 (57)	0.566
Digit span backward	7.07	(1.92)	7.12	(2.09)	7.00	(1.72)	0.239 (57)	0.812
TMT-A (s)	25.75	(8.44)	24.65	(8.28)	27.15	(8.61)	−1.129 (57)	0.264
TMT-B (s)	58.09	(18.93)	55.15	(18.03)	61.83	(19.72)	−1.355 (57)	0.181
Aversiveness rating US	8.14	(1.18)	8.06	(1.32)	8.23	(0.99)	−0.546 (57)	0.587

CGI: Clinical Global Impressions Scale; SIGH-A: Hamilton Anxiety Scale; PAS: Panic and Agoraphobia Scale; ASI: Anxiety Sensitivity Index; BDI II: Beck Depression Inventory II; TMT-A: Trail Making Test A; TMT-B: Trail Making Test B; US: unconditioned stimulus fear conditioning task.

[a] Available for $n=55$ patients.

lower dimensional representation using principal components analysis (PCA) as this reduces the dimensionality of the problem from the number of voxels to the number of samples (for details of this dimensionality reduction approach, see Mourao-Miranda et al. (2005)).

To ensure the generalizability of the classifier, we used leave-one-out cross-validation (LOO-CV) to predict a participant's comorbidity status. In each LOO-CV run, data from all but one sample (S-1 of the S subjects) is used to train the classifier. Subsequently, the categorization of the remaining subject, which has so far been unseen by the algorithm, was calculated. This procedure was repeated S times, each time leaving out a different subject, yielding each subject's categorization. Sensitivity was indicated by the number of true positives divided by the sum of true positives and false negatives, while specificity was indicated by the number of false negatives divided by the sum of false negatives and true negative cases. Accuracy was computed by summing sensitivity and specificity and dividing by 2.

To establish whether the observed classification accuracy was statistically significant, we ran each classifier 1000 times with randomly permuted labels and counted the number of permutations which achieved higher accuracy than the one observed with the true labels. The P-value was then calculated by dividing this number by 1000.

Comorbid patients showed higher age (and associated age of disease onset) and symptom load in the SIGH-A and (as a trend) in the PAS. In order to control for potential confounders, we conducted a second analysis using as input the residuals after regressing with age, SIGH-A, and PAS using matlab's "regress.m" function.

3. Results

3.1. Sample characteristics

PD/AG+DEP patients exhibited significantly higher BDI II scores and more comorbid disorders. They were slightly older than PD/AG−DEP patients and reported stronger symptom load on the SIGH-A. No differences were observed in neuropsychological characteristics (Table 1).

3.2. Group differences in fear conditioning

Results are given in Tables 2 and 3. During acquisition, a main effect of CS indicated successful fear acquisition (Fig. 1). Post-hoc contrasts showed that increased activation in the bilateral superior temporal gyri, insulae and right supplementary motor area was driven by enhanced responding to the CS+ unpaired compared to the CS− in the entire group. Neural conditioning effects were however not reflected by subjective ratings in valence and arousal ratings in the entire group (as indicated by a CS × time interaction;

Table 2
Brain activation clusters during fear conditioning and extinction in patients with panic disorder and agoraphobia with (PD/AG+DEP) or without comorbid depression (PD/AG−DEP).

Contrast/region	Side	Voxels	*x*	*y*	*z*	*F* or *t*	*P* uncorr.
Acquisition phase							
Main effect CS							
Superior temporal gyrus[a]	R	3339	64	−38	8	19.07	<0.001
Superior temporal gyrus	L	1339	−48	−14	0	15.55	<0.001
Insula	L	315	−40	8	−6	13.52	<0.001
Supplementary motor area	R	274	8	8	54	11.72	0.001
Post-hoc *t*-contrast: CS+ unpaired > CS−							
Superior temporal gyrus[a]	R	4639	64	−38	8	4.37	<0.001
Superior temporal gyrus	L	1097	−48	−14	0	3.94	<0.001
Insula	L	690	−40	8	−6	3.68	<0.001
Precentral gyrus	L	203	−26	−20	72	3.51	<0.001
Inferior frontal gyrus triang.	L	382	−52	28	26	3.42	<0.001
Supplementary motor area	R	455	8	8	54	3.42	<0.001
Post-hoc *t*-contrast: CS− > CS+ unpaired	No differential activation						
Main effect group							
Superior frontal gyrus	R	2227	24	34	38	13.82	<0.001
Post-hoc *t*-contrast: PD/AG−DEP > PD/AG+DEP							
Superior frontal gyrus	R	4552	24	34	38	3.72	<0.001
Putamen/insula[b]	R	193	26	18	6	3.24	0.001
Post-hoc *t*-contrast: PD/AG+DEP > PD/AG−DEP	No differential activation						
Interaction effect group × CS	No differential activation						
Extinction phase							
Main effect CS	No differential activation						
Main effect group	No differential activation						
Interaction effect group × CS							
Precuneus	R	371	4	−72	54	15.72	<0.001
Amygdala (ROI)	R	30	18	0	−16	13.84	0.005[c]
Middle frontal gyrus	R	247	28	26	46	13.84	<0.001
Hippocampus	L	174	−18	−34	−2	13.65	<0.001
Precuneus	R	192	10	−46	−6	12.87	<0.001
Precentral gyrus	L	150	−38	−16	58	12.79	<0.001
Middle cingulate gyrus	R	586	6	−36	32	11.54	0.001
Calcarine gyrus	L	162	2	−88	8	9.98	0.002
Post-hoc *t*-contrast: PD/AG+DEP > PD/AG-DEP (CS+ > CS−)							
Precuneus	R	2857	4	−72	54	3.96	<0.001
Amygdala (ROI)	R	47	18	0	−16	3.72	0.002[c]
Middle frontal gyrus	R	378	28	26	46	3.72	<0.001
Hippocampus	L	377	−18	−34	−2	3.69	<0.001
Precentral gyrus	L	261	−38	−16	58	3.58	<0.001
Middle temporal gyrus	R	194	66	−14	−12	3.54	<0.001
Thalamus	R	201	12	−26	0	3.45	<0.001
Inferior temporal gyrus	R	284	58	−48	−16	3.45	<0.001
Fusiform gyrus	R	317	30	−40	−18	3.37	<0.001
Inferior temporal gyrus	L	260	−54	−52	−20	3.31	<0.001
Cerebellum	L	202	−16	−76	−24	3.12	0.001
Post-hoc *t*-contrast:							
PD/AG-DEP > PD/AG+DEP (CS+ > CS−)	No differential activation						

L: left; R: right; CS+: stimulus associated with the unconditioned stimulus (unpaired); CS−: CS not associated with the unconditioned stimulus; voxel: number of voxels per cluster; *x*, *y*, *z*: MNI coordinates; $P < 0.005$ (uncorr.) with a minimum cluster size of 142 contiguous voxels, indicating to correct for multiple comparisons at $P < 0.05$. Region-of-Interest (ROI) analysis on the amygdala: $P < 0.05$ (FWE corr.) with a minimum cluster size of 10 continguous voxels.

[a] Cluster encompassing the right anterior insula (MNI coordinates *x*, *y*, *z*: 35, 20, −2).
[b] Cluster encompassing the right anterior insula (MNI coordinates *x*, *y*, *z*: 30, 20, −8).
[c] FWE corrected *P*-value.

valence:$F=1.827$, df=2, 112, $P=0.166$; arousal:$F=0.358$, df=2, 96, $P=0.700$) or as a function of comorbidity status (group × CS × time interaction; valence: $F=1.582$, df=2, 109, $P=0.211$; arousal: $F=1.210$, df=2, 96, $P=0.302$; Fig. 1).

On an neural level, the main effect of group during the acquisition phase indicated that PD/AG+DEP compared to PD/AG−DEP patients showed decreased activation in the bilateral dorsolateral prefrontal cortex (dlPFC) and a cluster encompassing the right putamen/anterior insula during the processing of both CSs (Fig. 2). Anxiety sensitivity was positively correlated with the magnitude of activation in the right anterior insula and superior frontal gyrus in the entire sample (Fig. 1, Table 3). No significant interaction of group and CS was observed during acquisition. During extinction, no differences in neural responding between the CS+ and CS− were observed, showing that the conditioned response was extinguished in the entire group. We further observed no group main effect, but the interaction effect of group × CS showed that processing the CS+ (signaling potential threat) and the CS− (safety cue) differed as a function of comorbidity. Post-hoc *t*-contrasts on this interaction effect specified only PD/AG−DEP patients who responded stronger toward the CS−, as indicated by enhanced activation in response to this safety cue in the right precuneus, middle frontal gyrus, middle temporal gyrus, fusiform gyrus, thalamus, amygdala, left hippocampus, precentral gyrus, cerebellum and bilateral inferior temporal gyrus (Fig. 3). Panic-specific symptomatology (PAS score) correlated significantly with the magnitude of amygdala activation toward the CS− in PD/AG−DEP patients only. No correlations with depressive symptoms were observed (Table 3).

Table 3
Association between brain activation and clinical symptoms (Pearson's correlations).

	All patients ($n=59$)			PD/AG−DEP ($n=33$)			PD/AG+DEP ($n=26$)		
	SFG	Insula	Amygdala	SFG	Insula	Amygdala	SFG	Insula	Amygdala
Panic symptoms									
PAS	−0.095	0.095	0.142	0.031	0.314	**0.392***	−0.085	0.007	−0.096
ASI	**0.269***	**0.349****	0.158	0.119	0.312	0.334	0.350	0.345	−0.127
Depressive symptoms									
BDI II	0.078	0.060	0.040	0.322	0.264	0.208	0.174	0.155	0.136

PAS: Panic and Agoraphobia Scale; ASI: Anxiety Sensitivity Index; BDI II: Beck Depression Inventory II. SFG: superior frontal gyrus (MNI: 24, 34, and 38); insula (MNI: 30, 20, -8); Amygdala (MNI: 18, 0, -16). Beta values from the right SFG and insula reflect the activation magnitude in response to both conditioned stimuli during the acquisition phase (contrast: PD/AG−DEP > PD/AG+DEP). Beta values from the right amygdala reflect the activation magnitude toward the CS− (safety cue) during the extinction phase (contrast PD/AG+DEP > PD/AG−DEP (CS+ > CS−)).

* $P<0.05$.
** $P<0.01$.

3.3. Prediction of comorbidity status

Comorbidity status was correctly predicted in 79% of patients (sensitivity: 73%; specificity: 85%; $P<0.001$) using a random undersampling tree ensemble (RUSTree Ensemble) in a LOO-CV framework based on the fear acquisition contrast. Using the main contrast during extinction, comorbidity status was correctly predicted in 73% of patients (sensitivity: 62%; specificity: 85%; $P<0.001$). Combining data from both conditions did not improve classification (accuracy: 73%).

When controlling for potential confounders, the accuracy nominally decreased, but still yielded above chance performance (acquisition contrast: accuracy: 73%; sensitivity: 77%; specificity: 70%; $P<0.001$; extinction contrast: accuracy: 62%; sensitivity: 50%; specificity: 73%; $P=0.035$). Combining data from both conditions under control of confounders nominally improved prediction accuracy (accuracy: 77%).

4. Discussion

Fear conditioning is a construct of core importance for the etiopathogenesis of PD/AG (Bouton et al., 2001). In particular, altered safety signal processing has been proposed in PD/AG (Lissek et al., 2009, 2010; Lueken et al., 2013, 2014). The present study pursued two major research aims. First, to characterize phenotype characteristics of comorbid depressive disorders in PD/AG patients; and second, to evaluate the potential of multivariate pattern recognition and machine learning in generating single patient predictions of comorbidity status based on neurofunctional activation during fear conditioning. Main findings are as follows: (a) altered safety signal processing as indicated by increased activation of fear circuitry functioning during the processing of learned safety cues was only present in patients without comorbid depression; (b) patients with comorbid depression deactivated dorsolateral prefrontal and anterior insular areas when processing conditioned stimuli; and (c) comorbidity status could be successfully predicted with fMRI data from the conditioning task with an accuracy rate of up to 79%.

Depressive disorders are the most frequent comorbidities in PD/AG (Kessler et al., 2006; Roy-Byrne et al., 2000). On a neurofunctional level, however, the impact of comorbid depression on the neural signature of primary PD/AG has not yet been examined, possibly due to the small sample sizes in prior neuroimaging studies that precluded subgroup analyses. Based on a comprehensive and well-characterized sample of PD/AG patients we here demonstrate for the first time that comorbid depression has a significant impact on the neural substrates of safety cue processing. These results extend findings from behavioral studies on comorbidity effects between anxiety and depressive disorders (Craske et al., 2012; Melzig et al., 2007) to the neural level and thus increase our understanding of the potential mechanisms underlying differences in safety signal processing as a function of comorbidity. Moreover, they shed some light on distinct aspects of the pathophysiology of these highly comorbid disorders. Being a cross-sectional study, conclusions regarding the development or causal relationships cannot be drawn. Future studies are nevertheless informed to anticipate comorbidity effects when studying behavioral and neural correlates of PD/AG and other anxiety disorders.

The neural signature of safety signal processing has been reported as an indicator of treatment response in PD/AG (Lueken et al., 2013). It was not present in patients exhibiting a current comorbid depressive disorder; however, response rates after CBT did not differ between patients with or without comorbidity, as we had shown in our previous analyses (Emmrich et al., 2012). In the present study, PD/AG+DEP patients showed stronger amygdalar activation in response to the CS+ than CS−. As fear conditioning is more closely related to research on anxiety than on mood disorders, only few studies on altered fear conditioning in depression are available. These, however, reported intact, if do not increased differential conditioning as indicated by increased skin conductance responses for patients with a major depressive disorder (Nissen et al., 2010), which parallel the present results on a behavioral level. A recent study on primary depressed patients with or without a comorbid panic disorder provided preliminary support for the role of hyperactive dorsal ACC functioning in reduced reward anticipation in major depression as a core pathophysiological feature of affective disorders (Gorka et al., 2014). They also indicated that comorbid anxiety may alter the association between MDD and neural responding to reward anticipation. These findings provide evidence that comorbidity effects between depressive and anxiety disorders may not be unidirectional.

Independent of the type of stimulus (threat vs. safety cues), we observed generally increased activation of the right anterior insula during fear acquisition in patients without comorbid depression. The insula has been associated with interoceptive processing (Craig, 2002; Seth, 2013); altered activation particularly in the anterior insula is a frequent finding in anxiety disorders (Etkin and Wager, 2007; Paulus and Stein, 2006) and subclinical anxiety proneness (Stein et al., 2007). In line, dimensional markers of anxiety sensitivity were correlated across both groups with the magnitude of insular activation. It has been reported by Lanius et al. (2007) that comorbid depression in PTSD patients attenuated

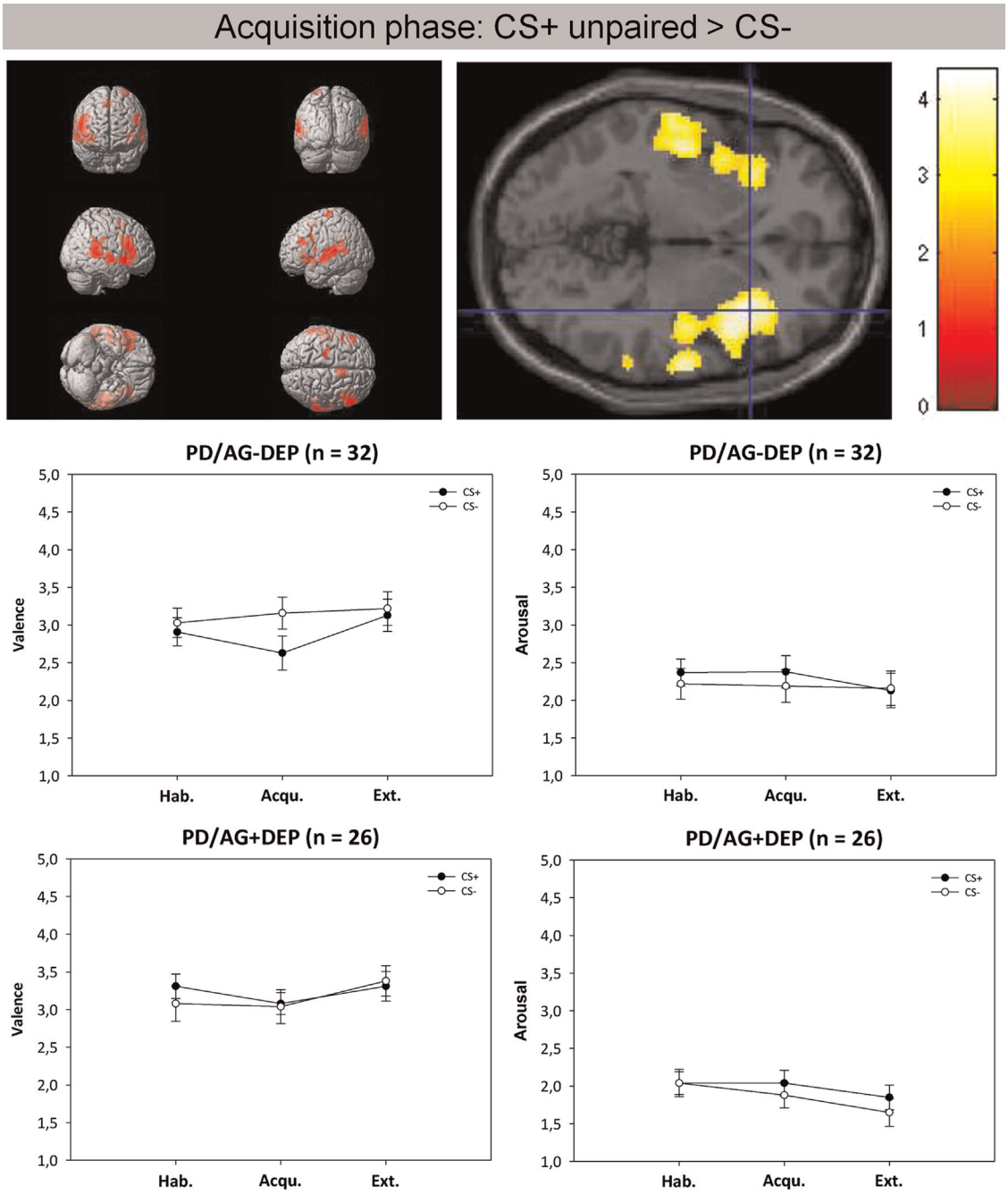

Fig. 1. Main conditioning effect (CS+ unpaired > CS−) in the acquisition phase for neurofunctional activation (upper half) and subjective ratings (lower half; self-assessment Manikin (SAM), with a 5-point Likert scale). Neurofunctional activation during CS+ processing encompasses the bilateral superior temporal gyri, bilateral anterior insulae (crosshairs at [35, 20, −2]), left inferior frontal operculum, bilateral inferior frontal gyri, and right supplemental motor area. Subjective ratings do not show a differential conditioning effect in neither of the two groups.

neural activation during trauma-script imagery in the left insula, further pointing toward the impact of comorbid depression on anxiety-specific neural activation patterns.

Comorbid patients also showed pronounced deactivation of the dlPFC during the processing of both CSs in the acquisition when compared to PD/AG patients without depression. In major depression, limbic–cortical dysfunctions have been proposed with relatively increased activation in ventral paralimbic, but decreased activation in dorsal neocortical streams, including the dlPFC (Mayberg, 1997). Of note, this functional imbalance resolved after deep brain stimulation of the subgenual cingulate (Mayberg et al., 2005). Current results on patients with comorbid depressive disorders appear to mirror these findings from primary major depression patients. Increased gray matter volumes in the left cingulate gyrus, right medial frontal gyrus, and left paracentral lobule have been reported for PD patients with comorbid depression (Kim et al., 2013), further supporting the notion of altered prefrontal functionality in depression. Experience of uncontrollable and unavoidable aversive stimuli during fear acquisition could surrender vulnerable patients to feelings of helplessness. As a central etiopathogenetic model for major depression, the concept of learned helplessness (Seligman, 1992) describes that repeated and uncontrollable experiences of aversive stimuli contribute to the development of this disorder. Learned helplessness is characterized by impaired motor escape responses, reduced motivation and learning deficits. Studying its

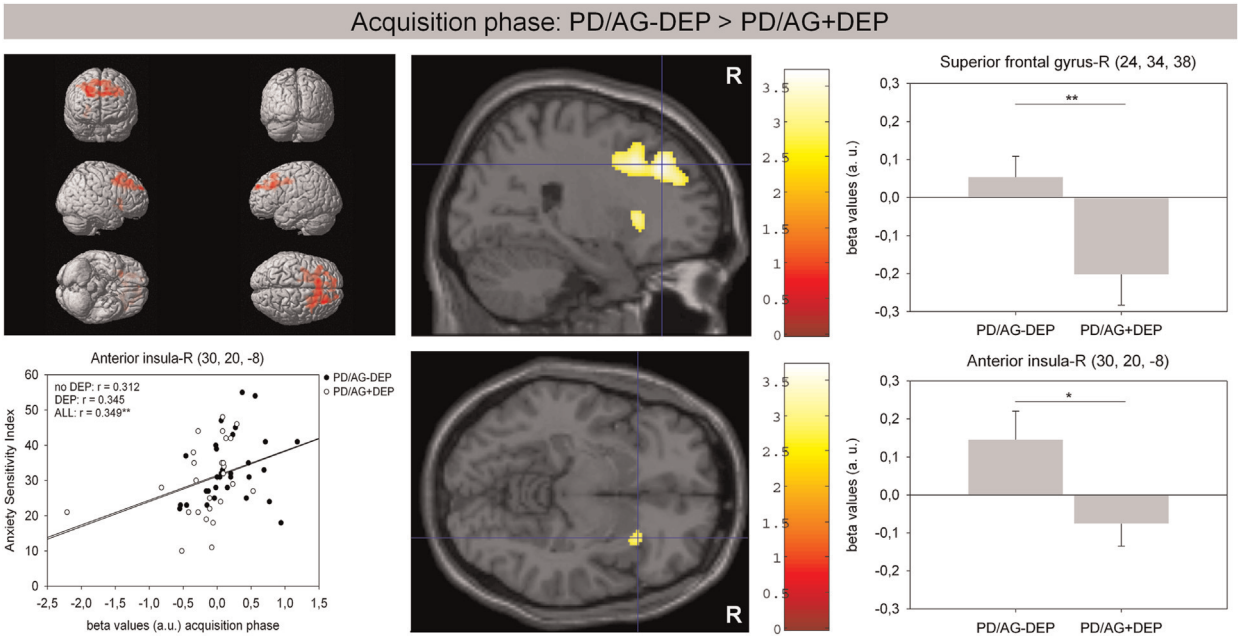

Fig. 2. Decreased brain activation in patients with comorbid depression (PD/AG+DEP) compared to patients without (PD/AG–DEP) in the bilateral superior frontal gyrus and right putamen/insula during fear acquisition (main effect of group). The scatterplot shows the positive association of anxiety sensitivity with the magnitude of neural activation in the right anterior insula in the entire group (correlation coefficients without outlier: PD/AG−DEP: $r=0.322$; PD/AG+DEP: $r=0.341$; combined group: $r=0.351^{**}$). R=right; $^{**}P<0.01$.

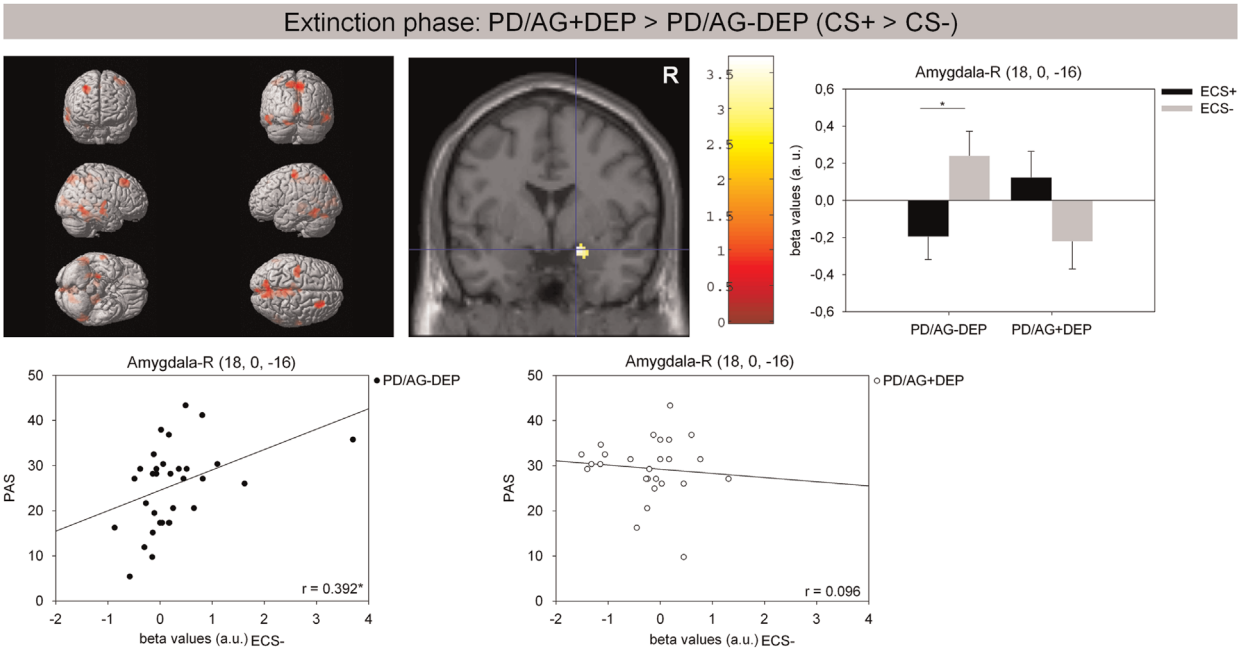

Fig. 3. Interaction effect group × CS during the extinction phase. Patients without comorbid depression (PD/AG−DEP) showed altered safety signal processing as evidenced by stronger responding in fear network structures toward the unreinforced conditioned stimulus (CS−) while the reverse pattern was observed in PD/AG+DEP patients. Scatterplots are given for the association between panic-specific symptomatology (Panic and Agoraphobia Scale; PAS) and the magnitude of neural activation in the right amygdala during CS− processing (correlation coefficient in the PD/AG−DEP group without outlier: $r=0.387^{*}$). R=right; $^{*}P<0.05$.

neurostructural substrates in chronic pain patients, Salomons et al. (2012) reported helplessness to be correlated with the cortical thickness in the supplementary motor area (SMA) and mid-cingulate cortex, regions implicated in cognitive aspects of motor behavior. Conversely, active avoidance behavior in a mouse model increased BOLD response in the hippocampal formation and in target regions of the hippocampus, such as the septum, nucleus accumbens, and anterior cingulate cortex/medial prefrontal cortex/motor cortex regions (Angenstein et al., 2013). In line with attenuated defensive reactivity in comorbid depressive patients (Melzig et al., 2007), altered cortically represented processing of stimuli during a threat situation could further substantiate the notion of learned helplessness, although we cannot provide direct behavioral measures for this hypothesis.

Multivariate pattern recognition and machine-learning approaches offer new ways to generate predictive information from neuroimaging data that can be used for single subject classificatory or prognostic purposes (Orru et al., 2012). They are thus well suited to complement mass-univariate analyses that focus on phenotype differences on a group level (Hahn et al., 2015; Lueken et al., 2015). We

here applied multivariate pattern recognition to a sample of PD/AG patients with or without depressive comorbidity to test its potential for classifying these groups solely based on functional MRI data. As one of the first studies addressing comorbidity status, we showed good accuracy rates in predicting whether a patient suffered from comorbid depression or not. In our data sensitivity rates outperformed specificity, a finding that is in accordance with the clinical need for sensitive screening instruments to detect psychiatric comorbidity. Accuracy rates were comparable to those of 80% obtained in a pattern analysis study differentiating unipolar from bipolar disorder (Grotegerd et al., 2014) based on amygdalar activation. Accuracies were numerically decreased when accounting for group differences in age, disease onset, and symptom severity (SIGH-A, PAS). These differences may however also reflect comorbidity status, as for example the SIGH-A is a relatively broad measure that substantially overlaps with depressive symptomatology such as sleep problems or autonomic symptoms. Controlling for these variables may thus likely yield too conservative results (Miller and Chapman, 2001). One could argue that the classifier picked up mainly features of symptom load, but groups were comparable on the CGI as an overall measure of disease severity, and after controlling for SIGH-A and PAS differences, the classifier still yielded acceptable accuracy rates. Present results show that based on neurofunctional data PD/AG with or without comorbid depression can be differentiated, a finding that could be of interest for clinicians in supporting individualized treatment planning or for other clinical entities where the differential diagnosis of anxiety and depression is less clear-cut as it is the case for generalized anxiety disorder (GAD). Future studies are encouraged to test this hypothesis on neuroimaging data in GAD vs. major depression.

4.1. Methods limitations

Findings should be interpreted in lights of the study limitations. We did not include a primary depressive patient group. Findings cannot be generalized to studies on major depression, as we solely focus on the impact of depression comorbidity in PD/AG. As such, we employed a functional paradigm tailored to the pathophysiology of anxiety disorders (i.e. fear conditioning). Although fear network components associated with the neural substrates of fear conditioning were successfully activated in the entire group, no conditioning effects were found on a subjective level. Valence and arousal ratings predominantly indicate declarative knowledge of contingencies, but not necessarily emotional learning. Due to methodological limitations in this multicentre study we were however not able to record autonomic markers as more proximal indicators of fear conditioning. Current findings suggest that anxiety-specific functional activation patterns are altered by comorbid depression. Since we did not include a depression-specific paradigm as an affective functional probe, we cannot delineate altered activation patterns that are specific for the comorbid group. Due to power considerations we collapsed patients with a current major depression and dysthymia; potential differential effects of these mood disorders need to be addressed by future studies. We studied the effect of currently present comorbidity. It is however possible that patients in the PD/AG – DEP group had experienced previous, yet fully remitted depressive episodes in the past, impacting on brain physiology in a however unknown way. As we studied a medication-free sample (also free of antidepressant medication) the presented findings are not biased by medication effects. Altered activation patterns may partly resolve under successful antidepressant medication, an issue that should be clarified by future research. The differential diagnosis of comorbid depression can be carried out with sufficient sensitivity and specificity based on subjective report. However, our approach was to provide a proof-of-concept that fMRI information can be used to predict comorbidity status of a single patient. Present results should stimulate future research to test the utility of neuroimaging data for less clear-cut constellations such as identification of at-risk subjects or prognostic questions where distinct clinical markers may not be available. As this is a first proof-of-concept on the single-patient classification of comorbidity status in PD/AG, validation in a second independent sample would be highly desirable. We included no healthy control group so that the present classifier was trained only to detect comorbidity within this patient sample, but not in mixed patient and control samples.

5. Conclusion

In conclusion, neural substrates during safety signal processing differed between patients with or without comorbid depression, a finding which may partly explain heterogeneous results across neuroimaging studies including comorbid patients (which are however representative for the typical patient seen in clinical practice). Furthermore, comorbid depression was associated with relative insular and dorsolateral prefrontal deactivation during the processing of cues signaling uncontrollable aversive stimuli, a finding which could reflect states of learned helplessness. Findings demonstrate the relevance of comorbidity patterns when investigating neurobiological substrates of anxiety disorders. The prediction of individual comorbidity status may translate neurofunctional data into clinically relevant information which might aid in planning individualized treatment.

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Conflict of interest statement

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Data access and responsibility

All principle investigators take responsibility for the integrity of the respective study data and their components. All authors and co-authors had full access to all study data. Data analysis and manuscript preparation were completed by the authors and co-authors of this article, who take responsibility for its accuracy and content.

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