Using novel imaging approaches in affective disorders : beyond current models

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Chapter 2
Aberrant limbic and salience network resting-state functional connectivity in panic disorder without comorbidity

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Abstract

Panic disorder (PD) is a prevalent and debilitating disorder but its neurobiology is still poorly understood. We investigated resting-state functional connectivity (RSFC) in PD without comorbidity in three networks that have been linked to PD before. This could provide new insights in how functional integration of brain regions involved in fear and panic might relate to the symptomatology of PD. Eleven PD patients without comorbidity and eleven pair-wise matched healthy controls underwent resting-state fMRI. We used seed regions-of-interest in the bilateral amygdala (limbic network), the bilateral dorsal anterior cingulate cortex (dACC) (salience network), and the bilateral posterior cingulate cortex (default mode network). RSFC of these areas was assessed using seed-based correlations. All results were cluster corrected for multiple comparisons ($Z > 2.3, p < .05$). Abnormalities were identified in the limbic network with increased RSFC between the right amygdala and the bilateral precuneus in PD patients. In the salience network the dACC demonstrated altered connectivity with frontal, parietal and occipital areas. The small sample size and hypothesis-driven approach could restrict finding additional group differences that may exist. Other caveats are reflected in the use of medication by two participants and the acquisition of the resting-state scan at the end of a fixed imaging protocol. We found altered RSFC in PD between areas involved in emotion regulation and emotional and somatosensory stimulus processing, as well as an area engaged in self-referential processing, not implicated in models for PD before. These findings extend existing functional neuroanatomical models of PD, as the altered RSFC may underlie increased sensitivity for bodily symptoms.

Introduction

Panic disorder (PD) patients experience recurrent unexpected panic attacks, followed by persistent concerns about having additional attacks, worrying about their consequences, and an associated change in behaviour (American Psychiatric Association, 1994). An influential neuroanatomical model of PD was proposed by Gorman and colleagues in 1989 (Gorman *et al.*, 2000). Central to their model is that panic derives from an abnormally sensitive fear network consisting of the prefrontal cortex, insula, thalamus, amygdala, and the amygdala's afferent and efferent projections from and to the hippocampus, brainstem, and hypothalamus. Further- more, a defective prefrontal cortical processing has been suggested to lead to misinterpretation of physiological triggers, ensuing in exaggerated amygdala and fear network activation, resulting in a panic attack (Gorman *et al.*, 2000, Shrestha *et al.*, 2009). Although this model has received considerable attention, the number of functional neuroimaging studies in PD is still modest (de Carvalho *et al.*, 2010). PET and SPECT studies of PD revealed decreased glucose use and/or blood flow in temporal and parietal areas, as well as in parts of the prefrontal cortex and (para)- hippocampal areas (Nordahl *et al.*, 1990, Lee *et al.*, 2006, Shin and Liberzon, 2010), while fMRI studies using a broad range of task paradigms reported activation and found altered activity in PD in cortical and limbic structures such as the anterior cingulate cortex (ACC), the amygdala, and hippocampus (de Carvalho *et al.*, 2010). The amygdala is perceived as the centre of the fear system with an important function in detecting, signalling, and learning from threat or danger (Phillips and LeDoux, 1992, LeDoux, 1998). Aberrant functioning of amygdala circuitry is thought to have a central role in the origin of PD and several other anxiety disorders (Phillips and LeDoux, 1992, LeDoux, 1998, Gorman *et al.*, 2000, de Carvalho *et al.*, 2010).

In contrast to task-evoked activity, resting-state fMRI enables examination of the brain's intrinsic functional connections in the absence of externally controlled stimuli or tasks (Biswal *et al.*, 1995, Fox and Raichle, 2007). Functional interactions between brain areas are crucial for proper functioning of the brain. This technique may therefore provide new insights in how functional integration of brain regions involved in fear and panic might relate to the symptomatology of PD (Fox and Raichle, 2007).

Consistently reported resting-state networks of potential relevance to PD include the default mode network (precuneus/posterior cingulate cortex (PCC), medial prefrontal cortex, and lateral parietal cortex), networks involving the amygdala, and the salience network (Damoiseaux *et al.*, 2006, Seeley *et al.*, 2007, Veer *et al.*, 2011). The salience network, comprising the dACC and bilateral anterior insula, is important in assessing the relevance of internal and external stimuli in order to guide behaviour (Seeley *et al.*, 2007).
Resting-state functional connectivity (RSFC) in PD has not been investigated before, in contrast to many other (neuro)psychiatric disorders (Broyd *et al.*, 2009, Greicius, 2008). In the present study we examined RSFC in patients with PD without comorbidity, using a seed-based correlation approach. Given the postulated model and the anatomical and functional abnormalities found in previous neuroimaging studies in PD, such as the frequently reported involvement of the amygdala circuitry and the ACC (Damsa *et al.*, 2009, de Carvalho *et al.*, 2010, Gorman *et al.*, 2000, Shin and Liberzon, 2010), we hypothesised that the amygdala-centred network would show altered connectivity of the amygdala with hypersensitivity of the fear circuitry and less top-down control. For instance, PD patients are known to be more aware of and to attribute a greater significance to signals coming from their own body than healthy controls. Specifically, we expected to find altered RSFC in networks involved in fear and emotion, and in distinguishing relevant from less relevant stimuli. For the salience network we expected a heightened awareness of bodily signals, i.e. increased connectivity of areas involved in somatosensory processing. As the default mode network shows altered connectivity in depression and other anxiety disorders (Broyd *et al.*, 2009, Fox and Raichle, 2007, Greicius, 2008), we also expected abnormalities in the connectivity of this network in PD.

Methods

Participants

All subjects were recruited from the MRI study from the large- scale longitudinal multi-centre cohort Netherlands Study of Depression and Anxiety (NESDA). NESDA is designed to investigate the long-term course and consequences of depression and anxiety disorders. NESDA participants were recruited from the community, through primary care and specialized mental health institutions. The rationales, methods and recruitment for NESDA have been described in detail elsewhere; for an overview of diagnostics,

inclusion and exclusion criteria see: (Penninx *et al.*, 2008, van Tol *et al.*, 2010).

After receiving written information, all subjects provided written informed consent. Participants underwent MRI in one of the three participating centres (Academic Medical Centre Amsterdam, Leiden University Medical Centre, and University Medical Centre Groningen) (van Tol *et al.*, 2010). The study was approved by the Medical Ethics Committees of all three centres.

For the present study on PD without comorbidity, resting-state fMRI data were available from 11 right-handed PD patients, and from 11 healthy controls pair-wise matched for age, gender, education, and scan-location (Table 1). All participants were new to lying in an MRI scanner. Patients were diagnosed with PD and no other psychopathology using the DSM-IV-based CIDI, lifetime version 2.1 (American Psychiatric Association, 1994). Participants were scanned within 8 weeks after the CIDI assessment. Severity of anxiety symptoms at baseline and at the time of scanning was measured with the Dutch version of the Beck Anxiety Inventory (BAI) (Beck *et al.*, 1988). Patients were excluded if they scored lower than seven on the Beck Anxiety Inventory, since they were then considered to have a 'minimal' level of anxiety and considered in remission (Beck *et al.*, 1988). Depressive symptoms on the day of scanning were rated with the Montgomery-Åsberg Depression Rating Scale (MADRS) (Montgomery and Asberg, 1979), as well as with the Inventory of Depressive Symptomatology (IDS) at baseline and at the time of scanning (Rush *et al.*, 1986).

Table 1. Demographic and Clinical Characteristics of Patients with Panic Disorder and Healthy Controls

			Panic Disorder patients(N=11) (N=11)	Healthy controls (N=11)		
Gender			1 male / 10 female	1 male / 10 female		
Scan location			3 AMC; 2 LUMC; 6 UMCG	3 AMC; 4 LUMC; 4 UMCG		
	Mean	SD	Mean	SD	*F* / *Z*	*p*
Age (years)	34.5	10.6	35.0	9.7	0.258[a]	0.974
Education (years)	12.8	3.5	14.1	2.1	-1.127[b]	0.260
BAI[†] score at scanning	14.5	5.6	1.9	2.5	-3.993[b]	0.001**
MADRS[‡] score at scanning	12.6	8.4	1.0	1.7	-3.776[b]	0.001**

AMC = Academic Medical Center Amsterdam; LUMC = Leiden University Medical Center; UMCG = University Medical Center Groningen; [†] BAI = Beck Anxiety Inventory; [‡] MADRS = Montgomery-Åsberg Depression Rating Scale; [a] *F*-value; [b] *Z*-value; ** Mann-Whitney U Test

Image data acquisition

Image acquisition took place at the three participating centres. Images were obtained on a Philips 3T magnetic resonance imaging system (Philips Healthcare, Best, The Netherlands), equipped with a SENSE-8 (Leiden University Medical Centre and University Medical Centre Groningen) or SENSE-6 (Academic Medical Centre Amsterdam) channel head coil.

As part of a fixed imaging protocol that also included task-related fMRI and structural MRI, resting-state functional MRI data were acquired for each subject using T_2^*-weighted gradient-echo echo-planar imaging with the following scan parameters in Amsterdam and Leiden: 200 whole-brain volumes; repetition time 2300 ms; echo time 30 ms; flip angle 80°; 35 transverse slices; no slice gap; field of view 220 × 220 mm; in-plane voxel size 2.3 × 2.3 mm; slice thickness 3 mm. Parameters in Groningen were identical, apart from: echo time 28 ms; 39 transverse slices; in-plane voxel size 3.45 × 3.45 mm. In the darkened MR room participants were instructed to lie still with their eyes closed and not to fall asleep. After completion of the scan, all participants confirmed wakefulness during acquisition. A sagittal 3-dimensional gradient-echo T_1-weighted image was acquired for registration purposes and grey matter analysis with the following scan parameters: repetition time 9 ms; echo time 3.5 ms; flip angle 80°; 170 sagittal slices; no slice gap; field of view 256 × 256 mm; 1 mm isotropic voxels.
No abnormalities were found upon inspection of the subjects' structural images by a neuroradiologist.

Data preprocessing

FMRI data processing was carried out using FEAT (FMRI Expert Analysis Tool) Version 5.98, part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl) (Smith *et al.*, 2004). The following pre-statistics processing was applied: motion correction; non-brain removal; spatial smoothing using a 6 mm full-width at half- maximum Gaussian kernel; grand-mean intensity normalisation of the entire 4D dataset by a single multiplicative factor; high-pass temporal filtering (Gaussian-weighted least-squares straight line fitting, with a 0.01 Hz cut-off). Registration of the RS data to the high resolution T_1-weighted image, and the T_1 to the 2 mm isotropic MNI-152 standard space image (T_1 standard brain averaged over 152 subjects; Montreal Neurological Institute, Montréal, QC, Canada) was carried out (Jenkinson

et al., 2002). The resulting transformation matrices were then combined to obtain a native to MNI space transformation matrix and its inverse (MNI to native space).

Statistical analysis

Demographic and clinical characteristics were analysed using SPSS 17.0 (SPSS Inc, Chicago, Illinois) using an independent-samples t-test with significance set at $p < 0.05$. If data did not meet the assumptions required to perform parametric analysis, the non-parametric Mann-Whitney *U*-test was performed.

For the current study, a seed-based correlation approach was employed to investigate functional connectivity during rest (Fox and Raichle, 2007). The following seed regions-of-interest were selected: bilateral amygdala (for the limbic network), bilateral dACC (for the salience network), and bilateral PCC (for the default mode network). We created a mask in standard space for the amygdala based on the Harvard-Oxford Subcortical Structural Probability Atlas in FSL (Veer *et al*., 2011). The coordinates for the dACC seeds were obtained directly from Table 1 of the study by Margulies and colleagues (Margulies *et al*., 2007), and posterior cingulate seed coordinates were obtained from the study by Greicius and colleagues (Greicius *et al*., 2003) (Table 3). Spheres of 4 mm radius were created around these seed voxels. The resulting masks were then transformed to native space by applying the inverse transformation matrix obtained from the registration procedure, and spatially averaged time series were extracted for each seed and for each subject. For each participant, and for each network of interest, we performed a multiple regression analysis using the general linear model (GLM) (as implemented in FEAT) (Smith *et al*., 2004). The time courses that were extracted from the voxels in our seed regions were entered as a regressor in a GLM for each network separately. Apart from the two regressors describing the left and right seeds, nine nuisance regressors were included in the model: signal from the white matter, cerebrospinal fluid signal, and the global signal, as well as six motion parameters (three translations and three rotations). The global signal was included to reduce artefacts associated with physiological signal sources (i.e. cardiac and respiratory) (Fox and Raichle, 2007).

After reslicing the resulting individual connectivity maps from our seeds and their corresponding within-subject variance maps into 2 mm isotropic MNI space, these were entered into a higher level within and

between groups mixed effects analysis (one- and two-sample t-test).
As several studies have identified structural abnormalities in PD (de Carvalho *et al.*, 2010), we used grey matter density information of each subject as a voxel-dependent covariate in our higher level model to rule out the influence of any subtle grey matter density variations. By including structural information in the functional connectivity analysis, variance explained by potential differences in grey matter density and/or possible misregistrations are taken into account (Oakes *et al.*, 2007). Lower level contrasts were analysed both within and between groups using the GLM in which age and scan location were also entered as regressors. To correct for multiple comparisons, cluster correction was applied in all group analyses with significance set at a corrected $p < .05$, using an initial cluster-forming threshold of $Z > 2.3$ (Worsley, 2001).

Results

Questionnaires

At the time of scanning, PD patients had a mean score of 14.5 (SD=5.6) on the BAI and 12.6 (SD=8.4) on the MADRS, scoring higher than controls on both scales (BAI 1.9; SD=2.5 and MADRS 1.0; SD=1.7, both p's < .05) (Table 1). BAI and IDS scores did not change significantly between baseline and time of scanning for PD patients (Table 2). Two out of eleven patients used an SSRI.

Table 2. Anxiety and Depression Severity at Baseline and Time of Scanning within Panic Disorder Patients

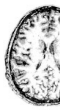
	Baseline		Scanning			
	Mean	SD	Mean	SD	*t* / *Z*	*p*
BAI[†] score	15.8	11.5	14.5	5.9	-.657[a]	.511*
IDS[‡] score	20.6	11.1	18.1	6.9	1.373[b]	.185**

[†] BAI = Beck Anxiety Inventory; [‡] IDS = Inventory of Depressive Symptomatology; [a] *Z*-value; [b] *t*-value;
* Wilcoxon Signed Rank Test; ** Paired-samples *t*-test

Table 3. MNI† Coordinates of the Seed Regions

Seed region	MNI coordinates		
	x	*y*	*z*
Amygdala	+/- 22	-6	-16
Dorsal Anterior Cingulate Cortex	+/- 6	18	28
Posterior Cinugulate Cortex/Precuneus	+/- 2	-52	26

† MNI = Montreal Neurological Institute



We first analysed amygdala RSFC. The seeds showed similar connectivity patterns in both groups encompassing the hippocampus, temporal poles, parahippocampal gyri, and the bilateral orbitofrontal cortex, consistent with previous literature (Stein *et al.*, 2007, Roy *et al.*, 2009). However, patients showed increased negative right amygdala connectivity compared to healthy controls with the bilateral precuneus and the bilateral lateral occipital cortex (Fig. 1, Supplementary Table 1). No group differences were found for the left amygdala analysis or when a contrast was made for the joint amygdala seeds.

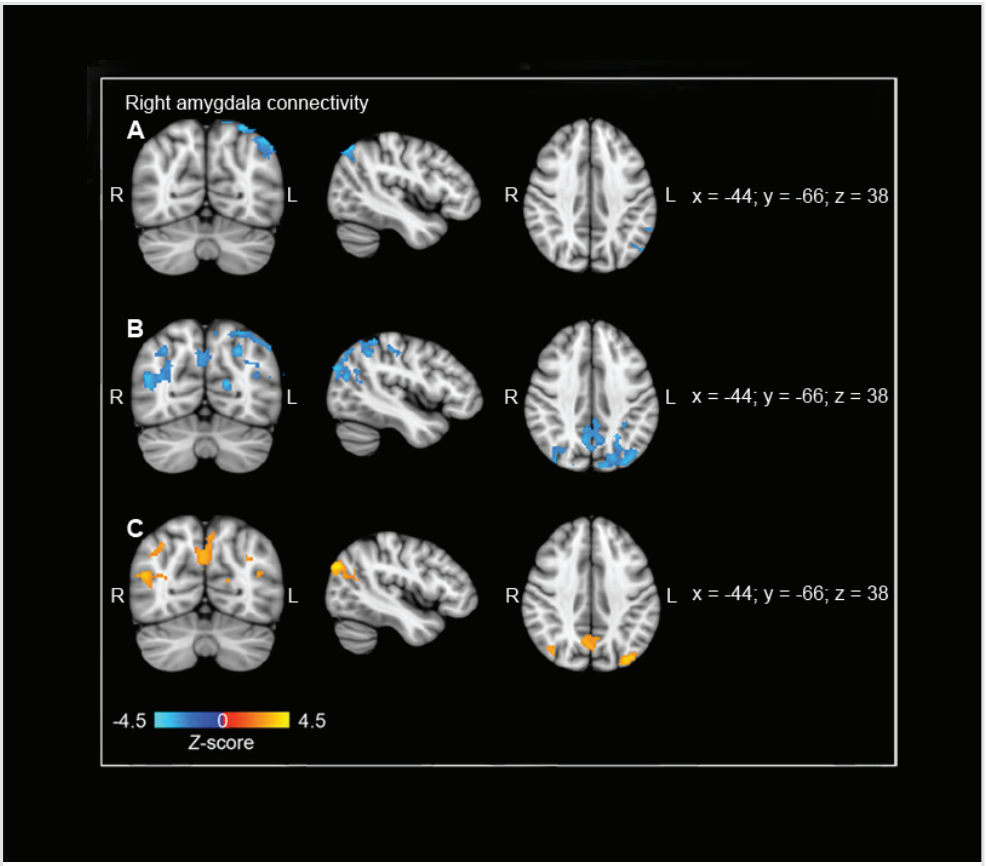


Figure 1.
Right amygdala negative connectivity. A=healthy controls; B=panic disorder patients; C=group difference: panic disorder patients > healthy controls. Results are cluster corrected at $p < .05$. Images are z-statistics, overlaid on the MNI-152 standard brain.

Next, we explored connectivity of the left and right dACC probing the salience network. Overall the seeds showed similar connectivity patterns in both groups (Figs. 2 and 3), corresponding with areas described in previous research (Margulies *et al.*, 2007). However, in PD the left dACC showed decreased connectivity with the bilateral frontal pole and superior/medial frontal gyrus compared to healthy controls.

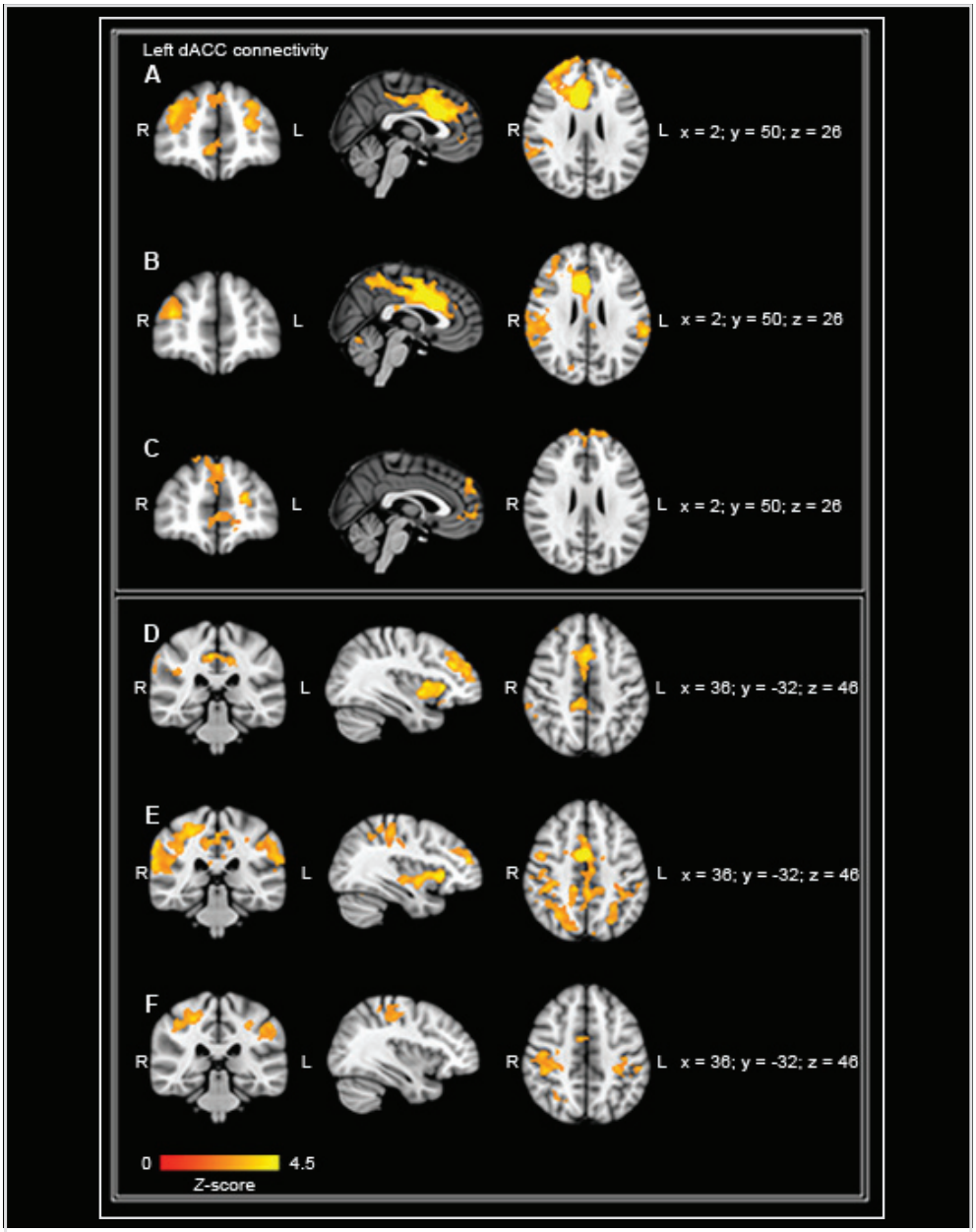


Figure 2.
Left dACC positive connectivity. dACC = dorsal anterior cingulate cortex; A = healthy controls; B = panic disorder patients; C = group difference: healthy controls > panic disorder patients D = healthy controls; E = panic disorder patients; F = panic disorder patients > healthy controls. Results are cluster corrected at $p < .05$. Images are z-statistics, overlaid on the MNI-152 standard brain.

In contrast, PD patients showed increased left dACC connectivity with the bilateral precentral and postcentral gyrus, the right supplementary motor cortex, and the right ACC (Fig. 2, Supplementary Table 2a and 2b). Finally, PD patients showed decreased right dACC connectivity with the right superior parietal lobule, the right lateral occipital cortex, the right angular gyrus, and the right central opercular cortex (Fig. 3, Supplementary Table 3). Combining the left and right dACC in one contrast did not produce any significant group differences.

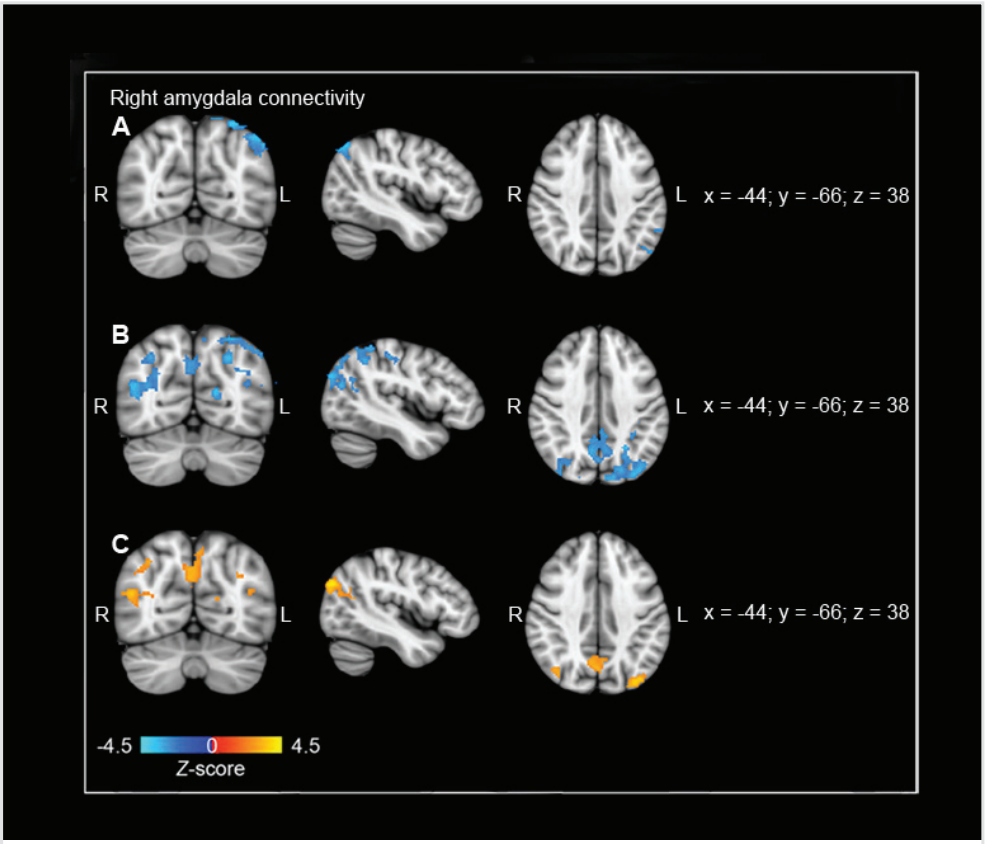


Figure 3.
Right dACC positive connectivity. dACC = dorsal anterior cingulate cortex; A = healthy controls; B = panic disorder patients; C = group difference A > B. Results are cluster corrected at $p < .05$. Images are z-statistics, overlaid on the MNI-152 standard brain.

Finally, we investigated connectivity of the default mode network with seeds in the bilateral PCC/precuneus, yet no group differences in default mode network connectivity were found.

Post-hoc, RS fMRI data were correlated with Beck Anxiety Inventory symptom scores using SPSS 17.0 (SPSS Inc., Chicago, Illinois, USA), to investigate whether the strength of connectivity was associated with symptom severity. Non-parametric tests were used if data did not meet the assumptions required for parametric testing. A mask was created of the resulting brain areas from our amygdala and dACC analyses, and the individual z-scores from these areas were calculated using Featquery, part of FSL (Smith *et al.*, 2004). No association was found between RS connectivity strength and anxiety scores.

Discussion

We investigated RSFC in PD patients without comorbidity and compared this to RSFC of pair-wise matched healthy controls with seeds in the limbic, salience and default mode network. As expected, abnormalities in connectivity were found in the limbic and salience networks, but no differences were observed in the default mode network. We found abnormal connectivity in PD patients between the right amygdala and the bilateral precuneus, as well as the bilateral lateral occipital cortex. We also found altered RSFC in patients between the left and right dACC on the one hand and frontal and more parietal and occipital areas on the other hand. Finally, we found abnormal RSFC in patients between the right anterior cingulate and the right superior parietal lobule, lateral occipital cortex, postcentral gyrus, and precentral gyrus.

Increased RSFC was found between the amygdala and precuneus in PD patients. The posterior cingulate/precuneus is thought to be involved in self-reflection and self-processing activities like mental imagery and episodic/autobiographical memory retrieval (Raichle *et al.*, 2001, Cavanna and Trimble, 2006b, Cavanna, 2007). Many of the functions of the precuneus and its connections seem of direct relevance to the phenomenology of PD. Four broad categories of cognitive-emotional functions have been assigned to the precuneus, namely visuo-spatial imagery, episodic memory retrieval, self-processing, and consciousness (Cavanna and Trimble, 2006b, Cavanna, 2007). Disturbances of self-processing and consciousness are characteristic elements of panic attacks

(American Psychiatric Association, 1994). Moreover, the precuneus and interconnected posterior cingulate and medial prefrontal cortices are constantly engaged in the gathering of information and representation of the self as well as the external world (Cavanna, 2007). The amygdala on the other hand serves as an important component of the system involved in the acquisition, storage, and expression of emotional and fear memory, playing a pivotal role in linking external stimuli to defence responses (LeDoux, 2000, LeDoux, 2003). Possibly, our results indicate that in PD patients, activation of the amygdala could lead to decreased functional connectivity with the precuneus. From the perspective of existing models, this finding might relate to decreased self-processing operations with typical symptoms such as depersonalization and loss of control experienced during a panic attack (American Psychiatric Association, 1994, Gorman *et al*., 2000, Cavanna and Trimble, 2006b). An alternative interpretation is that a disturbance in self-processing operations underlies the susceptibility to panic attacks, which would be in line with the continuously present feelings of unsteadiness, depersonalization and derealisation reported by many PD patients (American Psychiatric Association, 1994).

For the salience network seeds, the left and right dACC, we identified several differences in connectivity with frontal and occipito-parietal areas, which have been implicated in the pathophysiology of PD before (de Carvalho *et al*., 2010). These areas are involved in the processing of somatosensory information, attentional control and self-awareness (Bisley and Goldberg, 2010, Koechlin, 2011).

The left dorsal anterior cingulate showed increased positive connectivity in PD with the postcentral gyrus, known as the somatosensory cortex. This region receives proprioceptive and cutaneous input from the body (Nelson and Chen, 2008). Therefore, increased positive connectivity of the dACC with the postcentral gyrus in PD patients might be taken to reflect the increased processing of somatosensory stimuli in PD, resulting from or leading to misattribution of innocuous internal and external signals as potentially harmful. For both left and right dorsal anterior cingulate we found altered connectivity with (bilateral) superior parietal regions. Functional connectivity of the ACC with superior parietal regions has previously been reported in healthy subjects, and was suggested to be important for the maintaining of an internal representation of bodily states, a function clearly relevant to

PD (Gusnard *et al.*, 2001, Margulies *et al.*, 2007, Wolpert *et al.*, 1998). Deviations within this circuitry might contribute to an inaccurate internal representation and interpretation of the bodily state in PD patients. We also found decreased connectivity of the left dACC with the bilateral superior frontal gyrus in PD patients. These findings are in line with previous functional studies in PD, finding reduced blood flow or glucose use in the same areas (right superior frontal gyrus) (Eren *et al.*, 2003, Lee *et al.*, 2006). Interestingly, a study performed by Goldberg and colleagues (2006) showed superior frontal gyrus activity extending to the dorsal part of the anterior cingulate, when during an introspection task a sensory stimulus was slowly presented to participants after which they had to rate the emotional effect elicited by these stimuli (Goldberg *et al.*, 2006). When the task increased in speed and difficulty, no activation in the superior frontal gyrus was observed, leading the authors to conclude that the brain is able to 'switch off' self-awareness when it needs all its resources to carry out a difficult task (Goldberg *et al.*, 2006). Thus, dACC connectivity with the superior frontal gyrus could possibly play a role in the disturbed self-awareness in PD patients, who can typically experience feelings like loss of control, going crazy, derealisation, and depersonalization during panic attacks (American Psychiatric Association, 1994).

Abnormalities of the default mode network have been found in various neuropsychiatric diseases, like Alzheimer's disease, schizophrenia, epilepsy, autism, attention deficit/hyperactivity disorder, and depression (Fox and Raichle, 2007, Broyd *et al.*, 2009). The default mode network is associated with functions such as self- referential mental processing, social cognition and emotional processing (Broyd *et al.*, 2009). We did not find abnormalities in the default mode network, suggesting that processes relying on the default mode network are not affected in PD, but this might be due to our small sample size. However, even at lower thresh- olds no abnormalities in connectivity of the posterior cingulate were identified.

Limitations

There are several limitations important to note. An important notion is that RSFC data should be interpreted with caution, and any interpretation refers only to functional connectivity between brain areas as opposed to the (dys)function of a distinct brain region. Our sample size is relatively small and therefore some group differences may not have been detected.

Also, by opting for a hypothesis-driven approach and thus only exploring specific resting-state networks, abnormalities in other networks might have been overlooked. On the other hand, the use of a seed-based region-of-interest approach allows easier replication of our findings. Pooling data from different centres is another possible limitation. To account for this as much as possible, we have matched our groups based on scanning site and also added a confound regressor for site in our statistical model. A potential limitation is that patients were not diagnosed on the exact day of scanning but a few weeks earlier during the baseline interview. We did not formally assess whether additional psychopathology developed between baseline and scanning, but subjects indicated no major changes in their symptom patterns during a short clinical interview. Anxiety and depressive symptoms were again assessed on the scanning day and scores were not different from baseline. Another potential limitation is that no specific panic disorder scale was used, although the BAI predominantly contains panic-related items. Another limitation is that only one male subject in each of our groups was included, thus restricting the generalizability of the results. Furthermore, two of the included patients were using medication, so we cannot fully exclude possible confounding effects of medication use. Finally, our RS fMRI data were acquired at the end of a fixed imaging protocol (after completion of three task-related fMRI runs and the acquisition of an anatomical scan (scan sequence: Tol, word encoding, T1-weighted, word recognition, faces)), which could potentially have influenced RS connectivity (i.e. a spillover effect) with PD patients still showing aberrant connectivity while the stimuli were no longer present. Despite its limitations, this study also has some noteworthy strengths. This is the first study to investigate RSFC with MRI in PD. The major strength of this study is the inclusion of PD patients without any psychiatric comorbidity. In addition, most patients were not using psychotropic medication.

Our hypotheses regarding alterations in RSFC in PD patients were partly confirmed. As expected, aberrancies were found in amygdala and salience network RSFC. However, our results did not support the hypothesis that DMN RSFC was deviant in PD patients. In summary, our study shows altered RSFC in PD patients of areas involved in salience and emotion processing with areas engaged in self-processing and somatosensory processing, not implicated in current functional neuroanatomical models for PD. Although replication is warranted, disturbed functional connectivity in these circuits should be taken into account in future functional neuroanatomical models for PD.

Supplementary tables

Supplementary table 1. Right Amygdala Resting-State Connectivity, PD > HC

Region	Side	Z-value	p-value	MNI coordinates		
Cluster 1, 412 voxels				x	y	z
Lateral Occipital Cortex	R	3.54961	0.0004	46	-66	18
Lateral Occipital Cortex	R	3.15913	0.0016	40	-68	38
Lateral Occipital Cortex	R	3.14055	0.0017	34	-68	44
Lateral Occipital Cortex	R	3.12683	0.0018	42	-72	34
Lateral Occipital Cortex	R	2.80562	0.0050	52	-72	26
Lateral Occipital Cortex	R	2.73754	0.0062	36	-66	18
Cluster 2, 616 voxels				x	y	z
Lateral Occipital Cortex	L	4.18839	0.0001	-44	-78	32
Lateral Occipital Cortex	L	3.77343	0.0002	-34	-80	38
Lateral Occipital Cortex, BA 19	L	3.32952	0.0009	-34	-86	32
Angular Gyrus	L	2.95843	0.0031	-48	-60	20
Lateral Occipital Cortex	L	2.9051	0.0037	-48	-78	20
Lateral Occipital Cortex	L	2.88227	0.0039	-46	-72	24
Cluster 3, 1080 voxels				x	y	z
Posterior Cingulate Cortex	R	3.90197	0.0001	8	-44	14
Precuneus	L	3.62946	0.0003	-6	-52	12
Precuneus	R	3.38172	0.0007	14	-60	22
Precuneus	R	3.37847	0.0007	4	-64	34
Precuneus, BA 7	L	3.31899	0.0009	0	-68	38
Precuneus	L	3.29569	0.0010	-18	-62	12

PD = panic disorder patients; HC = healthy controls; MNI = Montreal Neurologic Institute; BA = Brodmann Area; voxel size 2 mm isotropic; z- and p-values of the local maxima, spread throughout the cluster.

Supplementary table 2a. Left dACC Resting-State Connectivity, HC > PD.

Region	Side	Z-value	p-value	MNI coordinates		
Cluster 1, 559 voxels				*x*	*y*	*z*
Frontal Pole, BA 9	L	3.5231	0.0004	-2	60	8
Frontal Pole, BA 10	L	3.59641	0.0003	-2	56	-2
Anterior Cingulate Cortex	R	3.57106	0.0004	8	42	-2
Frontal Pole	L	2.97862	0.0029	-8	66	12
Frontal Pole	L	2.82357	0.0047	-18	66	-4
Paracingulate Gyrus	R	3.3773	0.0007	0	50	2
Cluster 2, 856 voxels				*x*	*y*	*z*
Frontal Pole	L	3.84389	0.0001	-20	48	16
Frontal Pole	R	3.78664	0.0002	0	54	40
Frontal Pole	L	3.79932	0.0001	-18	58	12
Frontal Pole, BA 9	L	3.2182	0.0013	-18	62	28
Frontal Pole	L	3.30719	0.0009	-8	66	26
Frontal Pole	R	3.08149	0.0021	12	26	26

dACC = dorsal anterior cingulate cortex; HC = healthy controls; PD = panic disorder patients ; MNI = Montreal Neurologic Institute; BA = Brodmann Area; voxel size 2 mm isotropic; z- and p-values of the local maxima, spread throughout the cluster.

Supplementary table 2b. Left dACC Connectivity, PD > HC.

Region	Side	*Z*-value	*p*-value	MNI coordinates		
Cluster 1, 524 voxels				*x*	*y*	*z*
Anterior Cingulate Gyrus, BA 24	L	3.57718	0.0003	-2	0	36
Supplementary Motor Cortex, BA 6	L	3.28865	0.0010	8	-6	52
Anterior Cingulate Gyrus	R	3.50019	0.0005	2	0	38
Supplementary Motor Cortex, BA 24	R	3.22197	0.0013	6	-2	48
Anterior Cingulate Gyrus, BA 24	R	3.26338	0.0011	6	0	38
Anterior Cingulate Gyrus	R	3.1454	0.0017	8	-4	32
Cluster 2, 714 voxels				*x*	*y*	*z*
Supramarginal Gyrus, BA 40	L	3.09563	0.0020	-52	-32	44
Postcentral Gyrus	L	3.36605	0.0008	-46	-22	40
Superior Parietal Lobule	L	3.38798	0.0007	-36	-40	40
Supramarginal Gyrus	L	3.2304	0.0012	-4	-34	40
Postcentral Gyrus	L	3.36252	0.0008	-38	-22	46
Postcentral Gyrus	L	3.19629	0.0014	-36	-24	42
Cluster 3, 1477 voxels				*x*	*y*	*z*
Superior Parietal Lobule	R	4.0731	0.0001	18	-56	58
Postcentral Gyrus, BA 4	R	3.72298	0.0002	44	-16	44
Postcentral Gyrus	R	3.67095	0.0002	26	-32	52
Supramarginal Gyrus	R	3.5436	0.0004	40	-34	46
Supramarginal Gyrus	R	3.23672	0.0012	56	-22	42
Postcentral Gyrus, BA 3	R	3.27967	0.0010	32	-30	60

dACC = dorsal anterior cingulate cortex; PD = panic disorder patients; HC = healthy controls; MNI = Montreal Neurologic Institute; BA = Brodmann Area; voxel size 2 mm isotropic; *z*- and *p*-values of the local maxima, spread throughout the cluster.

Supplementary table 3. Right dACC Resting-State Connectivity, HC > PD

Region	Side	*Z*-value	*p*-value	MNI coordinates		
Cluster 1, 426 voxels				*x*	*y*	*z*
Precentral Gyrus	R	3.78293	0.0002	58	2	16
Central Opercular Cortex	R	3.37909	0.0007	52	2	4
Precentral Gyrus	R	2.78087	0.0054	52	0	16
Central Opercular Cortex, BA 43	R	3.06963	0.0021	58	-2	8
Central Opercular Cortex, BA 22	R	2.77364	0.0055	62	-4	6
Central Opercular Cortex	R	2.67973	0.0074	48	0	14
Cluster 2, 493 voxels				*x*	*y*	*z*
Superior Parietal Lobule	R	3.43418	0.0006	34	-56	52
Superior Parietal Lobule, BA 7	R	3.4361	0.0006	28	-56	54
Lateral Occipital Cortex, BA 7	R	3.27644	0.0011	20	-58	60
Angular Gyrus	R	2.89024	0.0038	38	-52	44
Superior Parietal Lobule	R	2.91071	0.0036	34	-42	56
Superior Parietal Lobule	R	3.08856	0.0020	18	-58	54

HC = healthy controls; PD = panic disorder patients; MNI = Montreal Neurologic Institute; BA = Brodmann Area; voxel size 2 mm isotropic; *z*- and *p*-values of the local maxima, spread throughout the cluster.