Chapter 2

rCBF differences between panic disorder patients and controls during anticipatory anxiety and rest


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Abstract

Background: Our goal was to identify brain structures involved in anticipatory anxiety in panic disorder (PD) patients compared to controls.

Methods: Seventeen PD patients and 21 healthy control subjects were studied with H215O PET scan, before and after a pentagastrin challenge.

Results: During anticipatory anxiety we found hypoactivity in the precentral gyrus, the inferior frontal gyrus, the right amygdala and the anterior insula in PD patients compared to controls. Hyperactivity in patients compared to controls was observed in the parahippocampal gyrus, the superior temporal lobe, the hypothalamus, the anterior cingulate gyrus and the midbrain. After the challenge, the patients showed decreases compared to the control subjects in the precentral gyrus, the inferior frontal gyrus and the anterior insula. Regions of increased activity in the patients compared to the controls were the parahippocampal gyrus, the superior temporal lobe, the anterior cingulate gyrus and the midbrain.

Conclusion: The pattern of rCBF activations and deactivations we observed both before and after the pentagastrin challenge was the same, although different in intensity. During anticipatory anxiety more voxels were (de)activated than during rest after the challenge.

Introduction

Panic disorder (PD) is a common and invalidating psychiatric disease. Several biological theories of PD have been proposed, including dysfunction of serotonergic and noradrenergic neurotransmitter systems, but till now the neurobiology of PD is unclear. Several groups have contributed to the discussion by providing data from animal studies, studies in humans using neurobiological challenges, post-mortem brain material, electroencephalogram (EEG), cerebrospinal fluid (CSF) and neuroimaging. At this moment different neuroimaging techniques are being used to further elucidate the role of different structures/neural networks in the brain involved in anxiety and panic.

Thus far, different neuroimaging techniques have been used to study PD. Structural neuroimaging (MRI) revealed abnormalities in brain structure in PD patients that correlated with the severity of PD, especially the temporal lobe abnormalities (Fontaine et al 1990; Ontiveros et al 1989).

Reiman et al (1984; 1986) were the first to report on functional neuroimaging in PD patients. They examined PD patients and controls with H15O PET scan after lactate challenge. They observed differences in the resting non-panic state between PD patients reacting with a panic attack on lactate challenge (lactate-positive), PD patients not experiencing a panic attack on lactate challenge (lactate-negative) and normal controls. In the right (more than in the left) (para)hippocampal region increases in rCBF were seen in lactate-positive patients compared to lactate-negative patients and controls. Nordahl et al (1990) have studied PD patients at rest using an auditory discrimination task with [18F]-2-fluoro-2-deoxyglucose (FDG) PET scan. They observed increases in glucose metabolic rate in patients compared to controls.
in the right hippocampal region and the orbitofrontal cortex. Decreases in patients compared to controls were seen in the anterior cingulate gyrus and the left inferior parietal lobule. Bisaga et al (1998) found similar results with FDG PET scan in 6 women with PD. They observed hyperactivity of the left hippocampal and parahippocampal region and hypoactivity of the right superior temporal lobe and the right inferior parietal lobe in patients compared to controls. Single-photon-emission computerized tomography (SPECT) studies showed similar results, although these studies were performed during or after lactate challenge (De Cristofaro et al 1993; Stewart et al 1988).

Since benzodiazepines possess anxiolytic effects, several groups have investigated whether changes in benzodiazepine receptor function were present in patients suffering from PD. One of these studies (Abadie et al 1999) observed no difference in binding potential, albeit this group not only included PD patients, but social phobia and generalized anxiety disorder patients as well. Others observed a decreased binding of the labeled compound to the benzodiazepine receptor in PD patients compared to controls. Overall global decreases were found in the studies of Malizia et al (1998) and Bremner et al (2000). Decreases in specific regions were found in the temporal lobe, in the inferior frontal region, the occipital region and the insula (Bremner et al 2000; Kaschka et al 1995; Kuikka et al 1995; Malizia et al 1998; Schlegel et al 1994). In a recent magnetic resonance spectroscopy study, Goddard et al (2001) observed reductions in total occipital cortex GABA levels. It is yet unclear if this abnormality is limited to certain cortical regions or present throughout the cortex, as this group only studied the GABA levels in a single occipital region of interest.

The results of the above mentioned neuroimaging studies are still inconclusive although hyperactivity of the parahippocampal gyrus seems to be one of the most consistent findings in PD patients. Based on data from clinical and preclinical studies we know that brain structures that are likely to be involved in anxiety are the prefrontal cortex, the anterior cingulate gyrus, the amygdala, the hippocampus, midbrain structures like the periaqueductal gray (PAG) and the locus coeruleus (LC), the hypothalamus and the (mainly dorsal) thalamus (for review see: LeDoux, 1998 and Davis, 1998).

We hypothesized that PD patients show increased anticipatory anxiety in the situation immediately before a pentagastrin challenge, and studied this in comparison with healthy control subjects. Furthermore, we studied the differences in brain activity between PD patients and healthy controls after the pentagastrin challenge at rest. Pentagastrin is a five-amino-acid long peptide very much alike to cholecystokinin-4 (CCK4). CCK, and pentagastrin have a high affinity for the so-called CCK\(_\text{B}\) receptors, the CCK receptors in the brain. These receptors may play a role in anxiety: neuroanatomical studies have revealed that CCK\(_\text{B}\) receptors are most abundant in the hippocampus (Beinfeld and Palkovits 1981; Dodd and Kelly 1981), there is an evident close relationship between CCK- and GABA-containing neurons in this brain region and CCK\(_\text{B}\) receptors have an excitatory influence on hippocampal pyramidal neurons, which can be completely abolished by benzodiazepines (Bradwejn and de Montigny 1984; Sinton 1988; Harro et al 1990). Both agents have been shown to reliably induce panic attacks and anxiety, especially in PD patients (de Montigny
The Anxious Brain

1989; Abelson and Nesse 1990; van Megen et al 1994). PD patients typically experience anticipatory anxiety before pentagastrin administration, expecting that they may have a panic attack during injection. In this study we used pentagastrin challenge to distinguish two states. The first state, before the pentagastrin was given, was hypothesized to be an anticipatory anxiety state, especially in the patient group. rCBF images were acquired during this state. Pentagastrin was then given, and a second state, after the effects of pentagastrin had subsided, was defined as a resting state. Resting state rCBF images were also acquired. We expected to see rCBF differences in brain regions that are known to play a role in the fear network in the brain.

Methods and Materials

Subjects

Seventeen patients with PD (5 men/12 women) and 21 healthy controls (9 men/12 women) participated in the study (for demographic data see table 1). All patients met the DSM-IV criteria of PD with or without agoraphobia, as diagnosed by a psychiatrist with a structured interview based on the DSM-IV criteria (American Psychiatric Association 1994). The subjects were physically healthy, as determined by history, physical and neurological examination, and had no comorbid psychiatric or neurological diseases (determined by a psychiatrist on the basis of a structured interview based on the DSM-IV diagnoses). All patients were free from psychoactive medication for at least three weeks prior to the PET scan (in the case of fluoxetine for at least 6 weeks). The patients were allowed to use oxazepam in the week before the PET scan, but not during the last 24 hours before the scan. The levels of anxiety and depression in the patients were obtained by the Hamilton rating scales for anxiety (Hamilton 1959) and depression (Hamilton 1967). The healthy control subjects had never experienced anything like a panic attack in their lives, and had no relatives with panic disorder or any other psychiatric disorder. All subjects were 18 years or older. None of the subjects was taking medication (other than oral contraceptives) or had a history of substance abuse. Other exclusion criteria were: DSM-IV mental disorder (other than PD in the PD patients), Hamilton depression rating scale score above 15, serious physical illnesses, radiological workers, participation in a trial with radioactive radiation exposure in the past year and pregnancy.

One of the male PD patients was excluded from the analysis because of too much movement during the scans. One male and two female PD patients experienced a spontaneous panic attack during the first scan; these three scans were excluded from the analysis. We choose to define a panic attack in this study with stricter criteria than stated in the DSM-IV: moderate to severe anxiety had to be present during the panic attack, in addition to the criterion of at least 4 out of the 13 symptoms, as described in the DSM-IV. Using this criterion sixteen of the seventeen PD patients had a panic attack during the pentagastrin challenge, while only two of the control subjects experienced a panic attack. None of the controls subjects experienced a panic attack either during rest after the challenge or in the anticipatory anxiety con-
dition before the challenge procedure.
All subjects gave oral and written informed consent after complete explanation of
the nature and possible consequences of the study (in accordance with the provisions
of the pertinent excerpt from the Declaration of Helsinki, South Africa 1996).
The study was approved by the medical ethics committee of the Groningen
University Hospital.

Procedure

Subjects were positioned in the scanner using a molded head rest with a strap over
the head to minimize head movement. Two marks were placed on the forehead and
nose of the subject to check for movement between the scans. The subjects were
instructed to lie quietly during the scan with their eyes open in a dimly lit room, with-
out conversation or noise, other than the noise from the scanner. They were not
allowed to speak, and no other person was present in the scanning-room during the
scan. They knew, however that a psychiatrist was watching via an infrared camera
and would be back in the room as soon as the 120-minute scanning time was over.
First a 20-minute transmission scan, used for attenuation correction, was made.
After this scan all subjects were scanned about 20 minutes before and 20 minutes
after a pentagastrin challenge (this was when the effects of the pentagastrin had
subsided). The pentagastrin was injected intravenously as a bolus injection of 40
µg, injected within 10 seconds. Immediately after each scan blood pressure (BP)
and heart rate (HR) were measured, and the subjects were asked about panic-rela-
ted symptoms experienced during the scan, with a semi-structured interview based
on the DSM-IV criteria for panic attacks.
Scanning was done between 11.30 am and 2.30 p.m., with a Siemens ECAT EXACT
HR+ whole-body PET camera operating in high sensitivity 3D mode. Subjects recei-
ved a total of 500 MBq of H215O per scan over 20 seconds through a forearm canula.
The scans were started at the moment of injection and lasted 120 seconds. Since
the time required for the radioactive water to reach the brain is between 20 and 25
seconds in the injection system used, the effective scanning time is 95-100 seconds.
Data are reconstructed by filtered backprojection with a zoom factor of 2.25. A
Hanning filter with a cut-off frequency of 0.5 cycles/pixel was used. The resulting
images have a nearly isotropic spatial resolution of roughly 5 mm FWHM (full width
at half maximum).

Data-analysis

The data were processed and analyzed with SPM software (SPM99, Wellicome
department of Cognitive Neurology, London, UK) (Friston et al 1995), implemented
in Matlab (Mathworks, Sherborn, MA, USA). The scans of all subjects were realign-
med and then normalized to the MNI (Montreal Neurological Institute) template
(using heavy regularization). Images were smoothed with a Gaussian kernel of 8
mm FWHM.
Activation differences in terms of hyper- and hypoactivity of patients compared to
control subjects were tested voxel-by-voxel by analysis of variance. Group differences
between patients and controls on the two scans were tested by a two sample t-test, while the overall differences were tested by a multigroup analysis, wherein both scans and both groups were compared. The multigroup analysis is named so within SPM to show that it is to be used for comparisons about group and state interactions, and the statistical test used in this analysis is an ANOVA. Clusters with voxel differences achieving a threshold of $Z = 3.15$ (corresponding to a p-value $< 0.001$ uncorrected) were displayed in three orthogonal projections on SPM glass brain projections. First the statistical parametric maps (SPMs) were inspected for the presence of findings in unpredicted regions; these were reported significant only if resisting correction for multiple comparisons based on the Gaussian random field theory ($p < 0.05$) (Friston et al 1996). Secondly the threshold was lowered to $P < 0.01$ uncorrected, and the SPMs were inspected for the presence of voxel clusters of statistical significance in anxiety-related regions where abnormalities were predicted a priori. Small volume correction was used to check if the differences detected in fear network related regions were significant. SPM99 uses the results of the formulae described in Worsley et al (1996) to calculate corrected statistics across the whole brain, by working out the shape and size of the whole brain volume in the analysis and calculating the correction accordingly. The stereotactic coordinates of the peak differences were determined using the Talairach and Tournoux atlas (1988), after translating the MNI coordinates to coordinates according to the Talairach template, by using the matlab function mni2tal.m, described by Brett (1999) on the website of the Cambridge Imagers Group (www.mrc-cbu.cam.ac.uk/imaging/mnispace.html). This function performs different linear transforms to different brain regions, taking into account that the Talairach brain is different and somewhat smaller in several areas than the MNI brain. The outcome of the matlab function is an estimate of the equivalent X, Y and Z coordinates in the Talairach brain.

Results

cBF differences between patients and controls during rest
(after the pentagastrin challenge)

Hypoactivity of patients versus control subjects was seen in the precentral gyrus left (trend, corrected $p = 0.07$) (figure 2-1; see colorfigure on page 119) and the insula left more than right. Hyperactivity of patients versus control subjects was seen in the parahippocampal gyrus, the left hippocampus, the right temporal lobe, the orbitofrontal cortex, the thalamus and the midbrain (figure 2-1; see colorfigure on page 119). The Z-scores and coordinates of peak voxels are presented in table 2.

cBF differences between PD patients and controls during anticipatory anxiety
(before the pentagastrin challenge)

Hypoactivity in PD patients compared to controls was seen in the precentral gyrus and the inferior frontal gyrus. Both clusters were more deactivated on the left side than
Table 1  **Demographic data and behavioral and physiological results**

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic Criteria</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean age (SD)</td>
<td>40.4 (13.8)</td>
<td>36.3 (13.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Mean no. panic attacks/2 wks (SD)</td>
<td>3.8 (3.9)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Total no. /5/12</td>
<td>5/12</td>
<td>9/12</td>
<td></td>
</tr>
<tr>
<td>Mean HAS (SD)</td>
<td>22.7 (5.1)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Mean HDS (SD)</td>
<td>9.9 (3.2)</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

**Behavioral and physiological results**

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean BP scan 1</td>
<td>137/85</td>
<td>128/76</td>
<td>NS</td>
</tr>
<tr>
<td>Mean BP scan 2</td>
<td>137/85</td>
<td>125/74</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Difference BP scan 1-2</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Mean HR scan 1 (SD)</td>
<td>75.2 (14)</td>
<td>68.8 (14.1)</td>
<td>NS</td>
</tr>
<tr>
<td>Mean HR scan 2 (SD)</td>
<td>73.8 (12.8)</td>
<td>62.7 (8.1)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Difference HR scan 1-2</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Mean N symptoms scan 1 (SD)</td>
<td>4.2 (3.3)</td>
<td>0.6 (1.2)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Mean N symptoms scan 2 (SD)</td>
<td>2.8 (2.7)</td>
<td>0.5 (1.4)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Difference symptoms scan 1-2</td>
<td>P &lt; 0.001</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

**Legend:** SD = standard deviation, HAS = Hamilton rating scale for anxiety, HDS = Hamilton rating scale for depression, BP = blood pressure, HR = heart rate, N = number.

on the right side (figure 2-2; see colorfigure on page 120). Furthermore the insula showed bilateral hypoactivity in patients compared to controls. The amygdala was significantly deactivated on the right side only in patients compared to controls, as shown in figure 2-3 (see colorfigure on page 121). Hyperactivity in patients compared to controls was seen in the parahippocampal gyrus (left more than right), the left hippocampus, the right temporal lobe, the orbitofrontal cortex, the anterior cingulate gyrus, the hypothalamus, the thalamus and the midbrain (figure 2-2; see colorfigure on page 120, and for the activation of the anterior cingulate gyrus figure 2-4 on page 121). The Z-scores and coordinates of peak voxels are presented in table 2.

**Multigroup comparison of rCBF differences between patients and controls on pre- and post-challenge conditions**

The multigroup comparison showed comparable results. Significantly increased activation was seen in patients compared to controls in the midbrain, the orbitofrontal cortex, the left anterior cingulate gyrus and the parahippocampal gyrus bilaterally. Significantly decreased activation in patients compared to controls was observed in the right amygdala and the left insula. The Z-scores and coordinates of peak voxels are presented in table 2.
Table 2  Regions where statistical significant differences in rCBF occurred between PD patients and healthy control subjects, the coordinates of maximal change, and the direction of change from rest to anticipatory anxiety

<table>
<thead>
<tr>
<th>Regions</th>
<th>Resting state anxiety</th>
<th>Anticipatory comparison</th>
<th>Multigroup</th>
<th>Direction of change from rest to anticipatory anxiety</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coord./Z-score</td>
<td>Coord./Z-score</td>
<td>Coord./Z-score</td>
<td></td>
</tr>
<tr>
<td>Hypoactivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Precentral gyrus left</td>
<td>-30 -13 41 (4.27)</td>
<td>-40 -9 47 (4.68)</td>
<td>More deactivated</td>
<td></td>
</tr>
<tr>
<td>Precentral gyrus right</td>
<td>38 -10 37 (5.36)</td>
<td>42 -8 39 (4.60)</td>
<td>More deactivated</td>
<td></td>
</tr>
<tr>
<td>Inferior frontal gyrus left</td>
<td>-55 14 7⁰ (3.10)</td>
<td>-50 7 24 (4.48)</td>
<td>More deactivated</td>
<td></td>
</tr>
<tr>
<td>Insula left</td>
<td>-42 -5 8⁰ (1.71)</td>
<td>41 -15 19 (3.20)</td>
<td>More deactivated</td>
<td></td>
</tr>
<tr>
<td>Insula right</td>
<td>38 -16 21 (3.32)</td>
<td>40 -4 35⁰ (2.34)</td>
<td>More deactivated</td>
<td></td>
</tr>
<tr>
<td>Amygdala right</td>
<td>24 3 -9⁰ (2.24)</td>
<td>22 -1 -10⁰ (2.22)</td>
<td>More deactivated</td>
<td></td>
</tr>
<tr>
<td>Hyperactivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parahippocampal gyrus left</td>
<td>-20 -57 -5 (3.26)</td>
<td>-20 -41 -5 (3.41)</td>
<td>More deactivated</td>
<td></td>
</tr>
<tr>
<td>Parahippocampal gyrus right</td>
<td>50 8 -36 (3.20)</td>
<td>48 8 -37 (3.33)</td>
<td>More deactivated</td>
<td></td>
</tr>
<tr>
<td>Temporal lobe right</td>
<td>-22 45 3⁰ (2.56)</td>
<td>-14 37 2 (3.19)</td>
<td>More deactivated</td>
<td></td>
</tr>
<tr>
<td>Anterior cingulate left</td>
<td>12 31 6⁰ (3.07)</td>
<td>12 29 -3 (3.33)</td>
<td>More deactivated</td>
<td></td>
</tr>
<tr>
<td>Anterior cingulate right</td>
<td>-4 61 -18⁰ (2.65)</td>
<td>-18 61 -15⁰ (2.21)</td>
<td>More deactivated</td>
<td></td>
</tr>
<tr>
<td>Orbitofrontal cortex left</td>
<td>2 59 -18⁰ (2.67)</td>
<td>24 56 -16⁰ (2.42)</td>
<td>More deactivated</td>
<td></td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>0 -6 -13⁰ (2.64)</td>
<td>0 -6 -13⁰ (2.64)</td>
<td>More deactivated</td>
<td></td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>2 -7 -16⁰ (2.98)</td>
<td>2 -7 -16⁰ (2.98)</td>
<td>More deactivated</td>
<td></td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>4 -5 -15⁰ (2.74)</td>
<td>4 -5 -15⁰ (2.74)</td>
<td>More deactivated</td>
<td></td>
</tr>
<tr>
<td>Basal ganglia</td>
<td>16 -1 9⁰ (2.23)</td>
<td>16 -1 9⁰ (2.23)</td>
<td>More deactivated</td>
<td></td>
</tr>
<tr>
<td>PAG/midbrain/pons</td>
<td>8 -31 -34⁰ (2.31)</td>
<td>-10 -26 -9⁰ (3.26)</td>
<td>More deactivated</td>
<td></td>
</tr>
<tr>
<td>Insula right</td>
<td>40 -15 17⁰ (2.23)</td>
<td>40 -15 17⁰ (2.23)</td>
<td>More deactivated</td>
<td></td>
</tr>
</tbody>
</table>

Legend: The coordinates of maximal change of the main voxels according to the Talairach and Tournoux atlas (1988), after translation of the coordinates via the function minjital (Brett, 1999); Z-scores presented are significant with a P < 0.05 corrected for whole brain statistics, * = after small volume correction

Differences in heart rate and blood pressure

The differences in heart rate (HR) and blood pressure (BP) between PD patients and control subjects were only significant after the resting scan (HR: F=10.4, df=1,36, p<0.01; BP: F=4.6, df=1,36, p<0.05), albeit the mean blood pressure and heart rate in patients both before and after the challenge were higher than in controls. There were no significant differences between the two conditions (table 1).

Differences in experienced panic-related symptoms between patients and controls

Significant differences were seen in the number and severity of the panic-related symptoms experienced by the PD patients and the control subjects (anticipatory anxiety: F=22.45, df=1,36, p<0.001; at rest: F=11.7, df=1,36, p<0.01) (table 1). The PD patients experienced on average four panic-related symptoms during the first PET scan. According to the DSM-IV criteria this is close to a panic attack. Two actual
panic attacks occurred in the PD patients during this scan (at least four panic-related symptoms together with a feeling of moderate to intense fear), while no actual panic attacks occurred in the healthy controls. The mean number of experienced symptoms in the patient group decreased significantly from anticipatory anxiety to at rest (F=23, df=1,15, p<0.001) (table 1). At rest no actual panic attacks occurred in the PD patients nor in the healthy control subjects.

**Discussion**

We observed rCBF differences between PD patients and control subjects in almost all regions proposed by LeDoux (1998) as being connected with the fear network in the brain. As was expected, in the resting state there were less anxiety related brain regions in both PD patients and control subjects, that showed activation differences than during anticipatory anxiety. The rCBF of PD patients during anticipatory anxiety differed from controls in the amygdala, the inferior frontal gyrus and the hypothalamus in the prechallenge condition, while differences in these areas were not detected in the resting state. The deactivation shown during anticipatory anxiety in the precentral gyrus and insula in patients compared to controls, was still present but less obvious in the subjects at rest. The PD patients in this study experienced a higher level of anticipatory anxiety than the normal control subjects, as shown by the higher number of experienced panic-related symptoms in the anticipatory anxiety condition, compared to the controls. They experienced a mean number of four DSM-IV described panic-related symptoms, which is the criterion for a panic attack according to the DSM-IV. Anticipatory anxiety has been associated with bilateral increases in the temporal poles (Reiman et al 1989a; Benkelfat et al 1995). The activity in these regions was also observed to be increased in other forms of anxiety (Reiman et al 1989b; Fredrikson et al 1993; Wik et al 1993; Rauch et al 1995; Fredrikson et al 1995). The data concerning the strong temporopolar hyperactivity in the studies of Reiman et al (1989a) and Benkelfat et al (1995) was suspected to be an artifact, when Drevets et al (1992) detected that these activation peaks were connected with extracerebral blood flow changes associated with teeth-clenching. Even though, the temporal lobe, the parahippocampal gyrus and the hippocampus seem to be implicated in anxiety and emotion regulation. Structural imaging studies discovered more abnormalities in the temporal lobe of PD patients than in normal controls (Ontiveros et al 1989); (Fontaine et al 1990). More recent neuroimaging studies also report temporal lobe activation in anxiety which is often unilateral and more medially located. We replicated this finding in the parahippocampal gyrus in PD patients compared to controls. This increase was especially present during anticipatory anxiety in the PD patients. In the resting condition the left parahippocampal region still showed more activity, although less voxels were activated, in the PD patients compared to the controls. Probably this region is not only related to anticipatory anxiety, but also to PD as an illness, as detected before by Nordahl et al (1990) and Bisaga et al (1998). Two recent EEG studies in healthy volunteers and social phobics have shown that anticipatory anxiety might be related to right-sided hyperactivity of the brain.
(Davidson et al 2000; Nitschke et al 1999), as compared to left-sided hyperactivity in state anxiety, e.g. related to panic (anxious arousal, Nitschke et al 1999). In our study the hyperactivity associated with anticipatory anxiety was located on both sides of the brain. This can be due to the fact that we studied PD patients instead of the healthy volunteers or social phobics who were included in the EEG studies. PD patients may have a higher state anxiety than healthy volunteers or social phobics. The precentral gyrus showed bilateral hypoactivity in patients compared to controls in the anticipatory anxiety condition. This is not easily explained, because if it would be associated with increased muscle tension, previously reported in anxiety and anticipatory anxiety states, the precentral gyrus should have been hyperactive. 

Another explanation for this hypoactivity might be that it is in fact comparable to the hypoactivity in the inferior parietal lobe and the superior temporal lobe seen in the studies of PD patients in rest of Nordahl et al (1990) and Bisaga et al (1998).

The inferior frontal gyrus plays a role in anxiety and emotion regulation. Stimuli leading to anxious arousal are processed in this area. The area is connected to the amygdala, which in its turn is seen as a key structure in the processing of anxiety in the brain (LeDoux 1998). The inferior frontal region is observed to be hypoactive during state anxiety in patients with anxiety disorders and in normal controls (e.g. induced by challenges) (Stewart et al 1988; Bremner et al 1997; Johanson et al 1998; Fischer et al 1998: unexpected panic attack; Javanmard et al 1999: 1 minute after CCK4 injection). Hyperactivity of the inferior frontal cortex has been observed in anticipatory anxiety in healthy volunteers and social phobics (Benkelfat et al 1995; Javanmard et al 1999). We detected anticipatory anxiety hypoactivity in PD patients compared to controls in this region. In this case, this hypoactivity can probably be attributed to the higher level of state anxiety in the PD patients compared to the control subjects, although it is strange that we could not detect any significant differences in this area after the challenge.

The amygdala is thought to play a key role in anxiety. LeDoux (1998) presents a model of anxiety processing, based on the results of studies on conditioned anxiety in rat and cat. The lateral nucleus of the amygdala receives projections from the sensory cortex (Inc. prefrontal cortex, anterior cingulate cortex) and the thalamus and processes this information to the central nucleus of the amygdala. The central nucleus of the amygdala projects to different areas in the hypothalamus and the midbrain, where responses are generated. Gorman et al (2000) proposed a model very similar to the above described model as a neurobiological model of PD. Different studies tried to show activation of the amygdala related to anxiety and fear. In studies wherein the recognition of facial expressions of fear was important, the role of the amygdala was shown (Adolphs et al 1994; Morris et al 1999). Two groups (Rauch et al 1996; Shin et al 1997) showed hyperactivity of the amygdala in subjects with posttraumatic stress disorder. Birbaumer et al (1998) showed amygdala activation during a facial recognition task in social phobics, using fMRI. This was the first study that showed activation of the amygdala in potentially fear-relevant stimuli. Other studies concerning anxiety failed to show activation of the amygdala (Chua et al 1999; Damasio et al 2000). An unilateral enlarged right amygdala was shown in the study of De Bellis et al in pediatric generalized anxiety disorder (2000). Adinoff et al showed an increase in the activity of the right amygdala in response to a pro-
caine challenge (2001). Unilateral hyperactivity of the left amygdala during anticipatory anxiety was found in the study of Phelps et al (1987). Cahill et al (2001) observed that during recall of emotionally related film-clips in women the left amygdala was activated, while in men the right amygdala was activated. This suggests a sex-specific effect in the activation of the amygdala. These different findings about the amygdala activation suggest that the amygdala plays a role in anxiety, although only amygdala activation was seen. In our study the right amygdala is deactivated in patients compared to controls, before the pentagastrin challenge. In the resting condition this hypoactivity could not be detected. It is possible that the hypoactivity of the right amygdala is specifically related to anticipatory anxiety in PD patients, and therefore seen only in the anticipatory anxiety condition. It is also conceivable that hypoactivity of the amygdala during anticipatory anxiety in PD patients is a sign of a specific functional impairment of the amygdala in PD patients only present during anticipatory anxiety. Another explanation may be that since somatosensory cortical areas as well as the prefrontal cortex exert inhibitory effects on the amygdala, it is conceivable that high anticipatory anxiety (which is cortically represented) reduces the activity of the amygdala.

The insula is a very differentiated area with postulated roles in the processing of visceral sensory, visceral motor, motor association, vestibular, somatosensory and language information. Projections to and from the thalamus, and projections to the prefrontal cortex, the anterior cingulate gyrus and the hypothalamus also suggest a role in the processing of anxiety-related information in the brain (LeDoux 1998; Gorman et al 2000). Especially the anterior insula is often mentioned in different studies about mood or emotion. An increase in activation in the insular region is associated with the induction of anxiety (Benkelfat et al 1995; Javanmard et al 1999; Rauch et al 1997; Reiman et al 1989b), and also with anticipatory anxiety (Chua et al 1999). The insula is furthermore considered as visceral sensory cortex to detect increased heart rate and visceral arousal, which is often part of anticipatory anxiety. In this study we detected lower activity in the anterior insula, both in the resting and in the anticipatory anxiety condition, in patients compared to controls. It is possible that this relative hypoactivity in the patients is actually hyperactivity in the control subjects as seen before in anticipatory anxiety in healthy control subjects awaiting a challenge situation (Benkelfat et al 1995).

Hyperactivity of the orbitofrontal cortex has been seen before in different anxiety disorders, such as obsessive compulsive disorder (Baxter et al 1987b; Schwartz et al 1996), simple phobia (Rauch et al 1995), posttraumatic stress disorder (Rauch et al 1996) and also in panic disorder (Nordahl et al 1990). Chua et al (1999) also found an increase in the anterior cingulate cortex activation during anticipatory anxiety in healthy volunteers. We observed an increase in activity in the anterior cingulate cortex in PD patients compared to controls both in the anticipatory anxiety condition and in the resting condition.
The Anxious Brain

We observed increased activity in the midbrain, probably the periaqueductal gray matter (PAG), in PD patients compared to control subjects, both before and after the challenge situation. Stimulation of midbrain areas in rats evoked anxiety-related behavior, and in humans a state of terror was evoked by stimulation of the midbrain (Nashold et al. 1969). The observed increase of activity in the midbrain is consistent with this finding.

In conclusion, in this study we observed hypoactivity during anticipatory anxiety in PD patients compared to controls in the precentral gyrus, the inferior frontal gyrus, the amygdala and the anterior insula. Hyperactivity in PD patients compared to controls was observed in the parahippocampal gyrus, the hypothalamus, the anterior cingulate gyrus, the basal ganglia and the PAG. In the resting condition only the precentral gyrus, the inferior frontal gyrus and the anterior insula were deactivated in the patients, and the parahippocampal gyrus, the anterior cingulate gyrus and the midbrain showed increased activity. Both before and after the pentagastrin challenge the differences between PD patients and healthy control subjects are seen in almost the same areas of the fear network in the brain. The main difference is that after the challenge, during rest, less voxels were activated than before the challenge, during anticipatory anxiety.

Of course, a limitation of this study is the fact that the scan made after the challenge is not a real resting situation, and may be influenced by the experienced challenge. Therefore it is still difficult to distinguish between the effects of anticipatory anxiety and the situation at rest. Even though, the experienced symptoms after the challenge show that the patients felt more relaxed than before the challenge, and that the healthy control subjects felt almost the same as before the challenge.

Another possible confounder can be that male and female subjects were taken together as one group. There are some, although contradictory, reports of differences between males and females in the mean total blood flow in the brain and lateralization of brain activity (Baxter et al. 1987a; Baxter et al. 1988; Cahill et al. 2001; Kastrup et al. 1999). Furthermore, no teeth-clenching scan was made for correction for facial muscle tension. We cannot completely control for the exact effect of teeth-clenching, but we assume that the observed temporal activation can not be attributed to muscle tension because the activation was more medially located and not bilateral.

In this study small volume correction was used for multiple regions that were expected to be connected with the representation of fear in the brain (LeDoux, 2000). The SPM99 output shows the regions P-values corrected for whole brain statistics, these statistics are run very conservatively, while it also shows the regions P-values not corrected for the total amount of statistical tests run, and these statistics are too liberal. There has been a lot of discussion on the SPM mailing list about whether to report the statistics with corrected (P < 0.05) or uncorrected (P < 0.001) P-values.

We chose to use the corrected P-values, but then statistically significant differences only emerge in very few areas. Thus we also used small volume correction, based on our expectation about the regions of the fear network, but these areas were only reported as far as they also showed an uncorrected P-value of less than 0.001.

Our conclusion is that we observed differences in brain activation between PD patients and controls in almost all areas previously hypothesized to be involved in anxiety. The differences in the precentral gyrus, inferior frontal gyrus, the (para)hip-
pocampal gyrus, the amygdala and the insula can be attributed to more anticipatory anxiety in the PD patients compared to the healthy controls. The activity in some of the regions, such as the insula, the inferior frontal cortex and the anterior cingulate cortex can possibly be a characteristic of anticipatory anxiety which is more chronically present in PD patients.