ORIGINAL ARTICLE

Altered Cerebral γ-Aminobutyric Acid Type A–Benzodiazepine Receptor Binding in Panic Disorder Determined by [^{11}C]Flumazenil Positron Emission Tomography

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Context: The benzodiazepine (BZD) receptor system has been implicated in the pathophysiologic mechanism of panic disorder (PD) by indirect evidence from pharmacological challenge studies and by direct evidence from single-photon emission computed tomography and positron emission tomography neuroimaging studies. However, the results of previous neuroimaging studies are in disagreement, possibly because of experimental design limitations related to sample size, matching between patients and controls, and confounding medication effects.

Objective: To compare BZD receptor binding between subjects with PD and healthy control subjects.

Design: Cross-sectional study for association.

Setting: Psychiatric outpatient clinic of the National Institute of Mental Health.

Participants: Fifteen subjects with PD who were naïve to BZD drug exposure and were not receiving other drug treatment, and 18 healthy controls.

Intervention: Images of BZD receptor binding were acquired using positron emission tomography and flumazenil tagged with carbon 11.

Main Outcome Measures: The BZD receptor binding potential was assessed by a simplified reference tissue-tracer kinetic model.

Results: The BZD receptor binding potential was decreased in multiple areas of the frontal, temporal, and parietal cortices and was increased in the hippocampus/parahippocampal region in subjects with PD vs controls. The most significant decrease was located in the dorsal anterolateral prefrontal cortex (DALPFC); the most significant increase, in the hippocampus/parahippocampal gyrus. These abnormalities were not accounted for by comorbid depression. In subjects with PD, the severity of panic and anxiety symptoms correlated positively with BZD receptor binding in the DALPFC but negatively with binding in the hippocampus/parahippocampal gyrus.

Conclusions: These data provide evidence of abnormal BZD–γ-aminobutyric acid type A receptor binding in PD, suggesting that basal and/or compensatory changes in inhibitory neurotransmission play roles in the pathophysiologic mechanism of PD. They also provide evidence of an impairment of frontal-limbic interaction in the modulation of anxiety responses, consistent with previous functional and structural neuroimaging studies in PD.

Arch Gen Psychiatry. 2008;65(10):1166-1175

PANIC DISORDER (PD) IS A COMmon pathological anxiety syndrome that may occur alone or in association with other psychiatric and medical conditions; in either case, the presence of PD increases the risks of disability and mortality.[1-3] The γ-aminobutyric acid type A–benzodiazepine ($GABA_A$-BZD) receptor complex has been implicated in the pathophysiologic mechanism of PD by various types of largely indirect evidence.[4] In healthy subjects, BZD receptor agonists show anxiolytic effects, and the BZD receptor inverse agonist FG-7142 induces severe anxiety that resembles panic attacks and the biological characteristics of anxiety.[5] In subjects with PD, some high-potency BZD receptor agonists exert antipanic effects. Nevertheless, subjects with PD (hereinafter referred to as PD subjects) show reduced neurophysiological sensitivity to BZD receptor agonists[6] and increased behavioral sensitivity to the anxiogenic properties of the BZD receptor antagonist, flumazenil.[7,8]

Neuroreceptor imaging studies of BZD receptor binding have reported abnormali-

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ties in PD, but their results are in disagreement. Some discrepancies across studies may be attributable to experimental design limitations involving the matching of control samples, presence of confounding medication effects, small sample sizes, and technical aspects of image acquisition and analysis. Using single-photon emission computed tomography (SPECT) and the selective BZD receptor ligand iomazenil labeled with iodine I 123, Brandt et al[9] found increased binding in 12 PD subjects relative to 9 healthy control subjects in the supraorbital prefrontal cortex (PFC). This finding was confounded by medication effects, however, because most subjects were receiving psychotropic drugs, including antidepressant agents known to alter GABAergic transmission.[10] Another SPECT-[^{123}I]iomazenil study compared BZD binding between 17 PD subjects who were receiving no medications and 17 healthy controls and reported an increased left-to-right radiotracer uptake in the PFC of the PD subjects.[11] A third SPECT-[^{123}I]iomazenil study reported that BZD receptor binding was decreased in the left hippocampus and precuneus, but increased in the caudate, cuneus, right dorsolateral PFC, and left middle temporal gyrus, in 13 PD subjects receiving no medication vs 16 healthy controls.[12] The specificity of this study was limited, however, by prominent differences in sex between the control and PD samples, the history of alcohol and/or other drug dependence (in 30%), and BZD exposure in the PD sample. Other SPECT-[^{123}I]iomazenil studies compared PD samples against control samples with epilepsy[13] or dysthymic disorder[14] but did not include healthy control (normative) samples.

Two studies assessed BZD receptor binding in PD using the more sensitive and higher-resolution technique of positron emission tomography (PET) and flumazenil tagged with carbon 11. In PD subjects naïve to BZD agonist drugs (n=7), Malizia and colleagues[15] reported a global reduction in BZD receptor distribution volume, with the most prominent reductions being evident in the right orbitofrontal cortex and insula in PD subjects relative to healthy controls. Cameron et al[16] found no significant difference in the global BZD binding, but confirmed the reduction in BZD receptor distribution volume in the insula in 11 PD subjects (most of whom were naïve to BZD drug exposure) relative to healthy controls, and observed that this finding was largely accounted for by the PD subjects with comorbid depression. In both PET studies, the interpretation of the results was limited by prominent imbalances in the sex ratio between the PD and control samples.

In summary, substantial indirect evidence exists of abnormalities of the central BZD receptor system in PD. Nevertheless, because of the inconsistency in the results and the methodological limitations of previous neuroreceptor imaging studies, the presence, location, and direction of abnormalities in BZD binding in PD remain unclear. The present study applied PET and [^{11}C]flumazenil to compare BZD receptor binding between PD subjects who were naïve to BZD anxiolytics and free of current medication effects and past substance dependence against a healthy control sample that was group matched to the PD sample for age and sex.

METHODS

SUBJECTS

Participants included 18 healthy controls and 15 volunteers who met *DSM-IV* criteria for current PD and had a Panic Disorder Severity Scale (PDSS[17]) score of more than 6. Subjects were recruited through advertisements posted in local newspapers and on the National Institutes of Health campus and underwent evaluation during screening visits in the outpatient psychiatry clinic of the National Institutes of Health Clinical Center. Psychiatric diagnoses were established using an unstructured interview with a psychiatrist (G.H.) and the Structured Clinical Interview for *DSM-IV*.[18,19] The family history of psychiatric disorders was assessed using the Family Interview for Genetic Studies.[20] The clinical evaluation also included a physical examination, electrocardiography, neuromorphological magnetic resonance imaging, and laboratory tests including liver and kidney function tests, hematology profile, thyroid function tests, urinalysis, and toxicologic (urine drug) screening. Behavioral ratings included the PDSS, the Hamilton Depression Rating Scale (HAMD), the Hamilton Anxiety Rating Scale (HAMA), the Panic Symptom Scale,[21] and the Beck Anxiety Inventory.

Exclusion criteria for all subjects included current medical or neurological disorders, lifetime exposure to BZD agonist drugs, exposure to psychotropic medications within 4 weeks of scanning (within 8 weeks for fluoxetine hydrochloride), lifetime history of substance use disorders, current nicotine use, pregnancy, and claustrophobia. An additional exclusion criterion for the PD subjects was having a current major psychiatric disorder other than PD, except major depressive disorder (MDD) and generalized anxiety disorder, which commonly occur comorbidly with PD.[1] Additional exclusion criteria for the controls included having a personal or first-degree family history of a major psychiatric disorder. After providing a full explanation of the study purpose and procedures, written consent was obtained as approved by the National Institute of Mental Health institutional review board.

POSITRON EMISSION TOMOGRAPHY

The PET images were acquired—as subjects rested with their eyes closed—using a scanner (GE Advance; GE Medical Systems, Waukesha, Wisconsin) with the septa retracted (35 contiguous sections; 4.25-mm plane separation; reconstructed 3-dimensional spatial resolution, 6- to 7-mm full width at half maximum). A transmission scan was acquired to correct for attenuation. After transmission scanning, a target dose of 20 mCi of high specific-activity [^{11}C]flumazenil was injected. The upper limit to the injection mass of [^{11}C]flumazenil was set at 9 μg/70 kg in all studies. A 60-minute dynamic emission image of the brain was initiated at injection. Subject motion correction during the PET acquisition was performed with a mutual-information registration of each scan's time frame to a standard frame before attenuation correction. Based on the calculated motion, the transmission images were resectioned and projected for final reconstruction and realignment.

To provide an anatomical framework for analysis of the PET images, structural MRIs were acquired using a 3.0-T scanner (Signa; GE Medical Systems) and a T1-weighted pulse sequence (radio-frequency pulses and rapid gradient-echo; voxel size, $0.9 \times 0.9 \times 1.2$ mm). The PET images were registered to the individual's MRI with a mutual information algorithm.

STATISTICAL ANALYSIS

Binding potential (BP) images were created using the 2-step version of the Simplified Reference Tissue Model (SRTM2).[22] In-

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Table 1. Demographic and Clinical Characteristics of the Study Sample

Characteristic	PD Subjects (n=15)	Controls (n=18)
Sex, No. F/M	11/4	13/5
Age, mean (SD), y	35.0 (11.6)	35.3 (11.3)
Age at onset of panic, mean (SD), y	23.6 (10.9)	NA
PD duration, mean (SD), mo	113 (130)	NA
Comorbid MDD, No. of subjects	8	0
Time spent in past major depressive episodes, mean (SD), mo	10.3 (7.5)	NA
Comorbid GAD, No. of subjects	3	0
Naïve to psychotropic drug treatment, No. of subjects	10	18
Family history, No. of subjects		
MDD	5	0
Bipolar disorder	1	0
Anxiety disorders	5	0
Alcohol dependence	6	0
PDSS score, mean (SD)	9.7 (3.0)	0.0 (0.0)
HAMA score, mean (SD)	11.3 (5.9)	1.4 (3.4)
Anxiety Sensitivity Index, mean (SD)	45.9 (13.8)	23.4 (4.3)
Panic Symptom Scale score, mean (SD)	27.6 (6.1)	13.1 (0.4)
HAMD score, mean (SD)	11.5 (7.3)	1.0 (1.7)

Abbreviations: GAD, generalized anxiety disorder; HAMA, Hamilton Anxiety Rating Scale; HAMD, Hamilton Depression Rating Scale; MDD, major depressive disorder; NA, not applicable; PD, panic disorder; PDSS, Panic Disorder Severity Scale.

put kinetics for the reference tissue were derived from the pons (delimited on the MR image), where [^{11}C]flumazenil binding predominantly reflects free and nonspecifically bound radiotracer.[23] The BP images (already transformed to magnetic resonance space) were then transformed to a common space (the Montreal Neurological Institute template) and statistical parametric mapping software (SPM2; Wellcome Department of Imaging Neuroscience, University College London, London, England). The PET images then were filtered using a 10-mm gaussian smoothing kernel to compensate for misalignment error arising during spatial normalization and individual anatomical variation.

Using the statistical parametric mapping software, we compared the [^{11}C]flumazenil BP values between groups in a voxelwise analysis using a 2-sample *t* test model. The statistical parametric map was thresholded at $P<.05$ uncorrected to facilitate the delineation of significant clusters. Voxels among the 3 most significant in each cluster, independently significant at $P\leq.001$ uncorrected, were reported. The *P* values were corrected for multiple comparisons using the cluster test and voxel-level familywise error tests,[24] and voxels where the differences remained significant after applying corrections were additionally indicated. The coordinates of each voxel were converted to the stereotactic spatial array of Talairach and Tournoux[25] using a linear transformation (http://imaging.mrc-cbu.cam.ac.uk/downloads/MNI2tal/mni2tal.m).

To assess the specificity of abnormalities in BZD receptor binding, post hoc voxelwise analyses were performed to separately compare the PD subgroups with and without a history of depression against the healthy control group and against each other. Because the statistical power for detecting differences relative to the controls was reduced in these smaller sample sizes, the significance threshold was lowered to $P<.005$ for these exploratory analyses. Age and sex were included as covariates in these subgroup analyses to adjust for differences in these parameters between subgroups.

In regions where differences in BP between the entire PD and control samples remained significant after applying corrections for multiple testing, the relationships between BP and panic, anxiety, and depression ratings were assessed post hoc. For each region, the BP values were mean centered and adjusted for baseline effects. Values from each PD subject were correlated with the PDSS, HAMD, and HAMA scores by computing Pearson product moment correlation coefficients using SPSS statistical software (SPSS Inc, Chicago, Illinois). Correlations with $r>0.5$ and $P<.05$ were reported in these exploratory analyses.

RESULTS

Table 1 shows demographic and clinical characteristics of the study samples. Of the PD subjects, 10 were naïve to psychotropic drugs and 5 had been medication free for a mean (SD) of 35 (35) months (range, 4-96 months). Eight PD subjects also had a history of MDD (mean [SD] age, 41.3 [11.0] years; 6 of them [75%] were women), whereas 7 denied ever meeting criteria for a major depressive episode (mean age, 27.9 years; 5 of them [71%] were women). One subject met criteria for PD with agoraphobia. No PD subject experienced a panic attack during PET scanning.

The mean (SD) [^{11}C]flumazenil dose administered did not differ significantly between the PD and control groups (19.7 [1.0] and 20.0 [0.6] mCi, respectively). The specific mean (SD) activity at the time of the injection was 1672 (493) mCi/μmol (range, 1036-2318 mCi/μmol) in the PD group and 1539 (322) mCi/μmol (range, 584-2134 mCi/μmol) in the control group ($P=.38$).

The PD subjects showed lower [^{11}C]flumazenil BP than did controls in the bilateral dorsal anterolateral PFC (DALPFC) (13% reduction on the right side at corrected $P<.05$), right frontal polar cortex, right dorsolateral PFC, bilateral precentral gyrus (19% reduction on the right side at cluster level, corrected $P<.001$), right postcentral gyrus, right superior temporal gyrus, anterior cingulate cortex, right superior parietal cortex, and left superior occipital gyrus (**Table 2**, **Figure 1**, and **Figure 2**). The PD subjects showed higher BP (by 16% on the right side and by 13% on the left side) than did controls in the hippocampus/parahippocampal gyrus bilaterally and the left dorsolateral PFC (Table 2, Figure 2, and **Figure 3**). The increase in the right and left hippocampus/parahippocampal cortex remained significant after correcting for multiple testing.

The post hoc subgroup analyses (**Table 3** and **Table 4**) showed that, relative to healthy controls, the subjects with PD alone and the PD subjects with MDD showed reduced BP in the DALPFC, bilaterally. The subjects with PD alone showed lower BP in the infralimbic cortex and higher BP in the right parahippocampal cortex than did the healthy controls and the PD subjects with MDD. The PD subjects with MDD showed higher BP than healthy controls and subjects with PD alone in the right middle temporal gyrus. Finally, the subjects with PD alone showed lower BP in the bilateral frontal polar cortex and left superior frontal gyrus and lower BP in the right inferior temporal gyrus, relative to healthy controls, but did not differ from the PD subjects with MDD in these regions.

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To correlate illness severity ratings with BZD receptor binding, the regional BP values in the DALPFC and the hippocampal/parahippocampal cortex were obtained for each PD subject by defining regional templates on the statistical parametric images. The right DALPFC template was defined from the parametric image for the contrast where the control BP is greater than the PD BP to encompass the voxel containing the peak *t* value (Table 2) and contiguous voxels in the same cluster for which the *t* value corresponded to $P<.001$. This template encompassed 466 voxels (3.73 mL) surrounding the peak voxel *t* value at x=21, y=49, and z=32. In this region, the BP values correlated positively with panic, anxiety, and depression severity ratings (PDSS: $r=0.70$ [$P=.006$]; HAMA: $r=0.59$ [$P=.03$]; HAMD: $r=0.56$ [$P=.04$]).

In the hippocampus/parahippocampal region, the BP values for individual PD subjects were obtained from templates defined on the contrast where the PD BP is greater than the control BP to encompass the peak voxel *t* value (right: x=30, y=−22, and z=−17; left: x=−26, y=−24, and z=−16) (Table 2) and the contiguous voxels in the same cluster for which the *t* value corresponded to $P<.005$ (the *t* values generally were smaller in these regions than in the right DALPFC [Table 2], so a lower statistical threshold was applied to encompass a comparable tissue volume). These templates encompassed 494 voxels (3.95 mL) and 283 voxels (2.26 mL) in the right and left hippocampus/parahippocampal regions, respectively. In these regions, BP correlated negatively with panic, anxiety, and depression severity ratings on the left (PDSS: $r=-0.59$ [$P=.03$]; HAMD: $r=-0.62$ [$P=.02$]; HAMA: $r=-0.54$ [$P=.049$]) and right sides (PDSS: $r=-0.62$ [$P=.02$]; HAMD: $r=-0.53$ [$P=.05$]; HAMA: $r=-0.55$ [$P=.04$]). Rating scales plotted vs mean centered and adjusted BP appear in **Figure 4**.

Finally, although sex-based subsamples were too small to assess sex differences within diagnostic groups for men, voxelwise analysis limited to women replicated the main findings from the entire sample. There was a similar reduction in [^{11}C]flumazenil binding in women with PD in the right precentral gyrus ($t=4.98$) and DALPFC ($t=4.21$). In addition, areas of elevated BP in women with PD vs control women were evident in the right ($t=4.58$) and left ($t=2.98$) parahippocampal gyri. Across the whole sample (PD plus control), no significant sex difference in [^{11}C]flumazenil BP was evident in the region of interest defined in the right DALPFC, right precentral gyrus, or bilateral parahippocampal gyrus over the areas where the groups had differed most in the initial voxelwise analysis.

Table 2. Brain Regions With a Difference in Receptor BPs of [^{11}C]Flumazenil Between PD Subjects and HCs[a]

Brain Region	x, y, z Coordinates	*t* Value
BP of HCs > BP of all PD subjects		
Right dorsal anterolateral PFC	20, 48, 27	5.74[b]
Right frontal polar cortex	30, 53, 8	4.31
Right dorsolateral PFC	36, 34, 20	3.42
Right precentral gyrus	32, −7, 50	4.52[c]
Right postcentral gyrus	44, −16, 34	3.72
Right superior temporal gyrus	65, −40, 8	4.12
Left precentral gyrus	−28, −17, 52	3.92
	−36, −5, 46	3.84
Left dorsal anterolateral PFC	−18, 42, 27	3.84
Anterior cingulate gyrus	−4, 26, 19	3.40
Left superior occipital gyrus	−26, −85, 19	3.72
Right superior parietal cortex	22, −68, 37	3.22
BP of all PD subjects > BP of HCs		
Right parahippocampal gyrus/ventral hippocampus	30, −22, −17	4.79[b,c]
Left parahippocampal gyrus/ventral hippocampus	−26, −24, −16	3.33[c]
Left dorsolateral PFC	−40, 39, 33	3.40

Abbreviations: BP, binding potential; [^{11}C]flumazenil, flumazenil tagged with carbon 11; HCs, healthy control subjects; PD, panic disorder; PFC, prefrontal cortex.

[a] Regions were derived from contrast analyses comparing the BP of [^{11}C]flumazenil between controls and PD patients. All results were significant at an uncorrected $P<.001$. Coordinates correspond to the stereotactic array of Talairach and Tournoux[25] and denote millimeters from the origin (anterior commissure), with positive x indicating right of midline, positive y indicating anterior, and positive z indicating dorsal to a plane containing the anterior and posterior commissures.

[b] Voxel level corrected *P* value significant at $P<.05$ after applying familywise error corrections for multiple testing.

[c] Cluster level corrected $P<.001$ after applying the cluster test for multiple testing.

COMMENT

The BZD receptor binding was decreased in several areas of the frontal, temporal, and parietal cortices but was increased in the hippocampus/parahippocampal region in PD subjects vs healthy controls. Comorbid depression did not account for these differences, based on post hoc comparisons involving PD subjects with no history of major depressive episodes. In PD subjects, the BP values and the severity of panic and anxiety symptoms were correlated positively in the DALPFC, but negatively in the bilateral hippocampus/parahippocampal gyrus.

Our results appear partly consistent with those of previous PET-[^{11}C]flumazenil studies that also selected PD cases who were naïve to BZD anxiolytics. Similar to our results (Table 2), Malizia et al[15] reported that BZD receptor binding was abnormally decreased in PD in areas of the frontal, anterior cingulate, temporal, parietal, and occipital cortices. Our finding that BZD binding was reduced in the right insula in PD subjects with comorbid depression vs healthy controls appeared compatible with the reports of Cameron et al[16] and Malizia et al[15] that BZD binding was abnormally decreased in the right insula in PD. Cameron et al showed that this difference was largely attributable to PD cases with comorbid depression.

In contrast to our results in the parahippocampal gyrus/ventral hippocampus, however, Cameron et al[16] observed no difference in this area, and Malizia et al[15] found reduced BZD receptor binding in a volume placed over the amygdala and adjacent hippocampus. Methodological issues may have contributed to these discrepancies. First, in our data the amygdala showed no evidence of abnormal BZD binding (Figure 3), and the affected area in the vicinity of the hippocampus localized to the interface between the parahippocampal gyrus and the anteroventral hippocampus; the volume assessed by Malizia et al may have diluted or missed this effect. Second, the prominent imbalance in sex ratios of the PD and control samples in these previous PET studies may have contributed to a type

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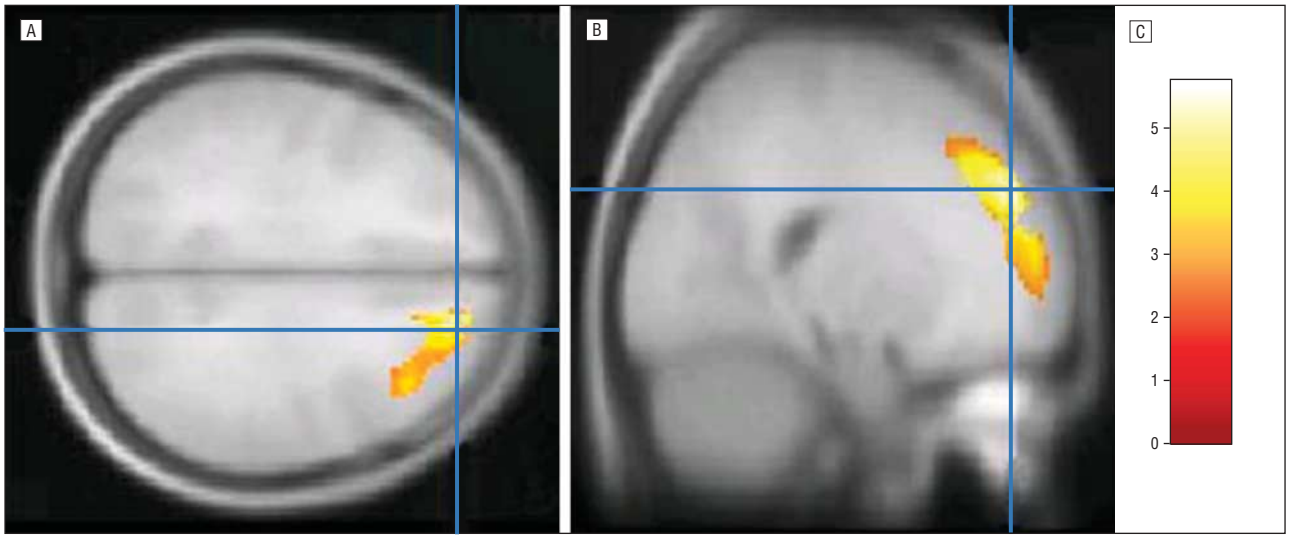

Figure 1. Statistical parametric map illustrating an area where benzodiazepine receptor binding was decreased in subjects with panic disorder vs control subjects in the right dorsal anterolateral prefrontal cortex. Voxel *t* values corresponding to $P<.01$ for the contrast of the positron emission tomography (PET) data between groups appear superimposed on horizontal (A) and sagittal (B) sections from an anatomical magnetic resonance imaging (MRI) template generated by averaging spatially normalized MRIs from multiple healthy subjects, as provided within the statistical parametric mapping software. The PET and MRI data have been spatially transformed to a common spatial array. The crosshair localizes the voxel containing the peak *t* value, located at x=20, y=48, and z=27 (interpreted as in Table 2). The color bar (C) indicates the magnitude of the voxel *t* values.

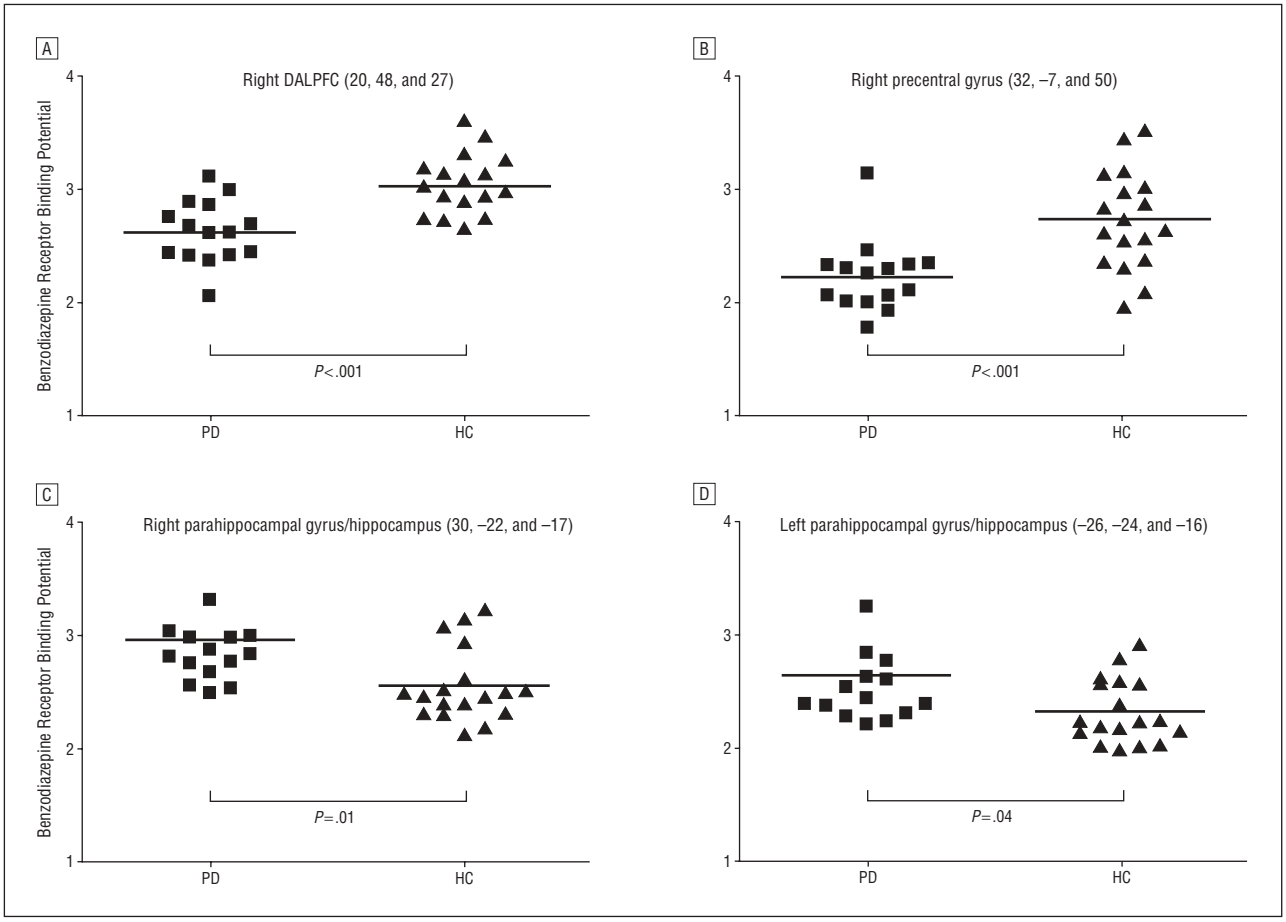

Figure 2. Scatterplots of individual benzodiazepine receptor binding potential (BP) of subjects with panic disorder (PD) and healthy controls (HC) in the right dorsal anterolateral prefrontal cortex (DALPFC) (A), the right precentral gyrus (B), and the right (C) and left (D) parahippocampal gyrus/hippocampus. Numbers in parentheses represent stereotactic coordinates x, y, and z, respectively. Horizontal lines indicate means. *P* values were derived from 2-tailed unpaired *t* tests. Means (SDs) of BPs in the 4 regions of interest were 2.6 (0.28) for the PD group and 3.0 (0.30) for the HC group in the right DALPFC; 2.2 (0.31) for the PD group and 2.7 (0.43) for the HC group in the right precentral gyrus; 3.0 (0.53) for the PD group and 2.6 (0.33) for the HC group in the right parahippocampal gyrus/hippocampus; and 2.6 (0.54) for the PD group and 2.3 (0.28) for the HC group in the left parahippocampal gyrus/hippocampus.

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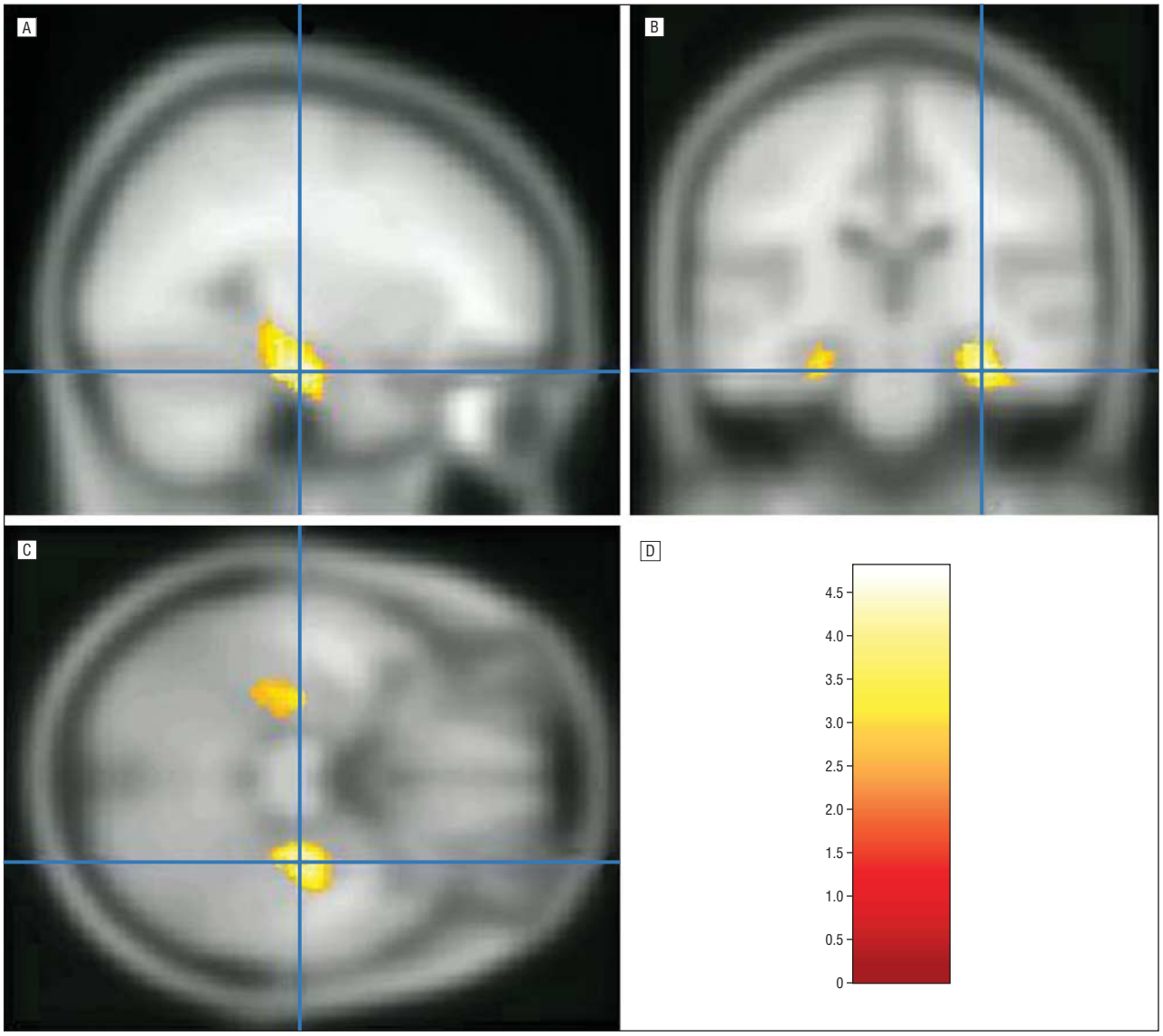

Figure 3. Statistical parametric map illustrating the areas where benzodiazepine receptor binding was increased in subjects with panic disorder vs control subjects in the hippocampus/parahippocampal region. The sagittal (A), coronal (B), and horizontal (C) sections are depicted. The interpretation of the voxel *t* values is described in the legend to Figure 1. The peak *t* value, indicated by the crosshair, is localized to x=30, y=−22, and z=−17, as interpreted in Table 2. The color bar (D) indicates the magnitude of the voxel *t* values.

II error (in the study by Malizia et al,[15] 3 of 7 patients were female whereas all 7 controls were male; in the study by Cameron et al,[16] 9 of 11 patients were female whereas 8 of 21 controls were female), although no study has been sufficiently powered to assess sex effects on BZD receptor binding in PD. Third, the sample sizes in these studies were small; thus, the failure to replicate results across studies may reflect a type II error, particularly because our post hoc assessments suggested that the elevated BZD receptor binding in the hippocampus/parahippocampal region was specific to PD subjects without comorbid depression (each of the previous PET studies included only 6 of such cases). The conservative image analysis approach of Cameron et al[16] further increased the chance of a type II error.

With respect to SPECT-[^{123}I]iomazenil studies, our findings appear compatible with those of Kuikka et al,[11] who reported increased left-to-right radiotracer uptake in the PFC of PD subjects; we found abnormally reduced [^{11}C]flumazenil binding in several right PFC areas and increased binding in a left dorsolateral PFC area in PD. In contrast, our results appeared to disagree with those of Bremner et al,[12] who reported decreased BZD binding in the left hippocampus and increased binding in the right dorsolateral PFC. Nevertheless, the peak difference in BZD receptor binding reported in the hippocampus in their study was situated 2 cm medial and dorsal to the area where we found abnormally increased [^{11}C]flumazenil binding (eg, the stereotactic coordinates for the hippocampal abnormality reported by Bremner et al[12] were x=12, y=−26, and z=−4, which localizes to the midbrain in Talairach and Tournoux[25]). Moreover, most of the PD subjects studied by Bremner et al were experiencing panic attacks at the time of scanning, raising the possibility that state-dependent changes in BZD receptor binding, excessive movement during imaging, or a lower specific-activity radioligand dose accounted for the

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Table 3. Brain Regions With a Difference in Receptor BPs of [^{11}C]Flumazenil Between 7 Subjects With PD Alone and HCs[a]

Brain Region	x, y, z Coordinates	*t* Value
BP of HCs > BP of subjects with PD alone		
Right frontal polar cortex	28, 51, 10	4.55
Right dorsal anterolateral PFC	20, 48, 23	3.95
	18, 33, 35	3.67
Left frontal polar cortex	–28, 51, 3	3.61
Left dorsal anterolateral PFC	–22, 42, 22	3.37
Left superior frontal gyrus	–16, 16, 45	2.41
Infralimbic cortex	–8, 11, –17	2.89
	4, 9, –16	2.85
BP of patients with PD alone > BP of HCs		
Right parahippocampal gyrus/ventral hippocampus	22, –26, –14	3.58
Right inferior temporal gyrus	40, –66, 0	2.81

Abbreviations: BP, binding potential; [^{11}C]flumazenil, flumazenil tagged with carbon 11; HCs, healthy control subjects; PD, panic disorder; PFC, prefrontal cortex.

[a] Regions were derived from contrast analyses comparing flumazenil BP between patients and controls. The models included age and sex as covariates. Coordinates are described in Table 2. All results were significant at uncorrected $P < .005$.

Table 4. Brain Regions With a Difference in Receptor BPs of [^{11}C]Flumazenil in 8 PD Subjects With Comorbid Major Depression and HCs[a]

Brain Region	x, y, z Coordinates	*t* Value
BP of HCs > BP of PD subjects with major depression		
Right dorsal anterolateral PFC	20, 52, 23	4.21
Left dorsal anterolateral PFC	–18, 52, 23	2.89
Right precentral gyrus	30, –11, 50	3.73
Left precentral gyrus	–26, –22, 58	3.03
Right insula	38, –23, 9	2.93
Right superior parietal cortex	20, –58, 43	2.88
BP of PD subjects with major depression > BP of HCs		
Left medial parietal cortex	–12, –34, 52	2.84
Right middle temporal gyrus	42, –36, –4	3.06
BP of subjects with PD alone > BP of PD subjects with major depression		
Right postcentral gyrus	30, –28, 46	3.62
Right parahippocampal gyrus	20, –28, –15	2.97
BP of PD subjects with major depression > BP of subjects with PD alone		
Right middle temporal gyrus	42, –36, –4	3.45
Left posterior orbital cortex	–14, 12, –17	3.28
Infralimbic cortex	–2, 9, –4	3.13

Abbreviations: BP, binding potential; [^{11}C]flumazenil, flumazenil tagged with carbon 11; HCs, healthy control subjects; PD, panic disorder; PFC, prefrontal cortex.

[a] Regions were derived from contrast analyses comparing flumazenil BP between patients and controls. The models included age and sex as covariates. Coordinates are described in Table 2. All results were significant at uncorrected $P < .005$.

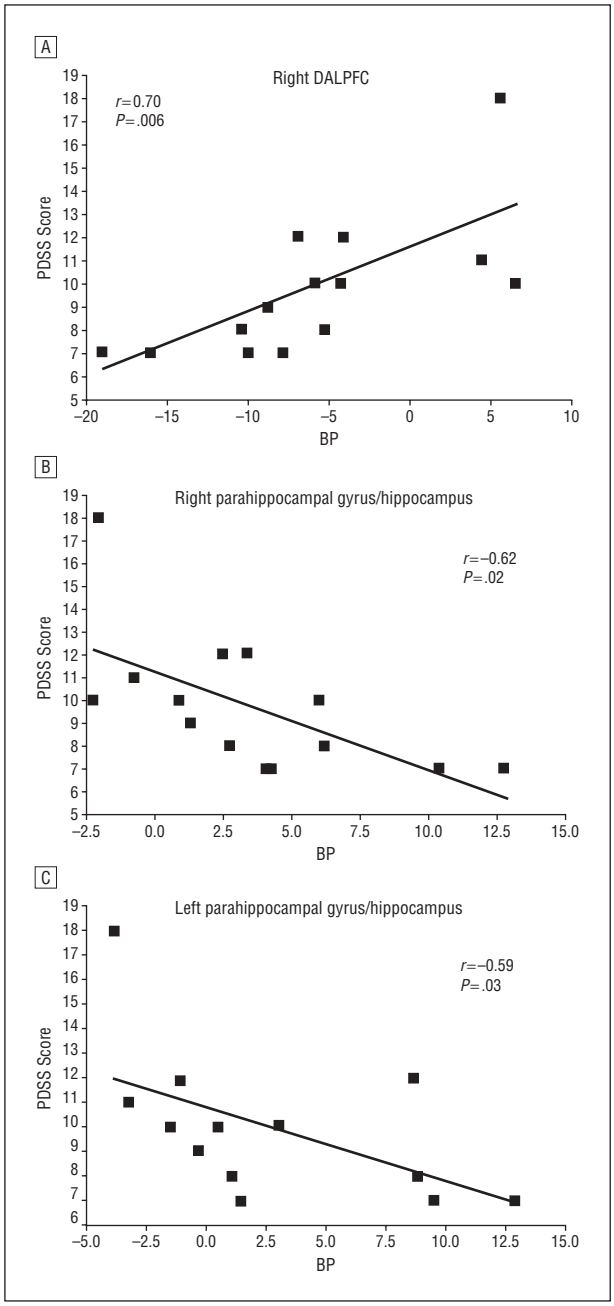

Figure 4. Scores on the Panic Disorder Severity Scale (PDSS) plotted against mean centered, adjusted benzodiazepine receptor binding potential (BP) in right dorsal anterolateral prefrontal cortex (DALPFC) (A) and right (B) and left (C) parahippocampal gyrus/hippocampal regions of interest. The regression lines were determined from the Pearson correlation coefficients and are included with the *P* values.

dissimilar results across studies. Bremner et al[12] also included subjects with histories of BZD use and alcohol and/or other drug dependence, whereas we excluded such cases, and their PD and control samples were not matched for sex. Finally, SPECT affords lower sensitivity and spatial resolution than PET does, limitations that would have reduced the specificity of measures in small structures such as the hippocampus.

Although most previous studies did not find correlations between anxiety symptom scores and BZD receptor binding,[9,11,15,16] 1 study reported a negative correlation between PFC BZD receptor binding and Panic Attack Symptom Scale scores.[26] Another study of subjects with various anxiety disorders (most had PD) showed a positive correlation between lateral and medial PFC BZD re-

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ceptor binding and Spielberg State Anxiety scores.[27] These findings appear compatible with ours regarding the direction of correlation in the DALPFC, although they used a state anxiety measure and we used a score of panic severity during the past 1 month.

Strengths of our study included the relatively large sample size, the exclusion of subjects with current psychotropic drug use or lifetime history of BZD exposure, the relatively close matching of PD and control samples with respect to age and sex, the relatively higher spatial resolution and sensitivity afforded by the PET-[^{11}C]flumazenil technique, and the improved accuracy of spatial transformation and localization achieved via PET-MRI coregistration. Moreover, we limited the injected mass of flumazenil to less than 9 µg/70 kg, and the specific activities of the [^{11}C]flumazenil injected in our study (mean values exceeded 1500 mCi/µmol) were higher than those used in previous studies of PD. Lassen et al[28] reported that a 10-mCi injection of [^{11}C]flumazenil at a specific activity of 450 Ci/mmol (0.02 µmol or 7 µg) resulted in a BZD receptor occupancy of 0.7% at 30 minutes. Based on these data, our injected mass of less than 9 µg/70 kg would have occupied less than 1% of brain BZD receptors.

The high prevalence rate of comorbid MDD in our PD sample was consistent with that of community studies demonstrating strong associations between depression and panic.[29] Moreover, the sample size allowed evaluation of the specificity of the findings in PD with respect to comorbid depression.

Several limitations of our methods merit comment. The sensitivity of our results may have been reduced by the inability to exclude healthy controls with a latent vulnerability to PD. In addition, our PD sample overrepresented women compared with the sex ratio of PD in the general population,[1] which may reduce the generalizability of the results. In addition, our experiment's cross-sectional design could not distinguish whether abnormalities in BZD receptor binding reflected a biological vulnerability for PD or a consequence of illness.

Finally, to obviate the need for arterial cannulation, we applied a simplified reference tissue model for obtaining BP. Simplified reference tissue modeling approaches with the pons as the reference tissue were validated against more invasive approaches that used arterial plasma input functions for deriving [^{11}C]flumazenil BP.[23,30] The BP values obtained using these 2 approaches were highly correlated (r=0.96 to r=1.00).[23,30]

The pons proved superior to the other reference regions tested for estimating free and nonspecifically bound [^{11}C]flumazenil with respect to the robustness of model fits, reliability, and lower bias.[27,30,31] Nevertheless, a moderate level of [^{11}C]flumazenil binding in the pons is receptor specific.[23,32] A limitation of applying reference tissue models for comparing [^{11}C]flumazenil binding between groups, therefore, is that a difference in regional BP may reflect abnormal BZD receptor binding in the region of interest or abnormal distribution volume in the pons. To address this issue, we relied on the consistent finding from previous studies of sampled arterial blood that the BZD receptor distribution volume in the pons did not differ significantly between healthy control and PD samples[12,15,16] or mixed anxiety-disordered samples composed predominantly of PD cases.[27] Nevertheless, because the 2-step version of the Simplified Reference Tissue Model ratios tracer binding in target tissues to that in the reference tissue, differences in the pons distribution volume between groups could influence the results in 1 direction only and thus could not account for abnormal increases in some regions and abnormal decreases in other regions in PD.

Furthermore, although the pons is relatively small, partial volume effects on a volume of interest of this size (range, 4.49-8.25 mL) are minor in images of 6- to 7-mm spatial resolution. The spherical properties of the pons also limit partial volume effects due to contamination with cortical gray matter. The ability to obtain measures from the pons with relatively little partial volume effect has been considered an advantage of this reference region over others assessed in the literature.[30] Nevertheless, any potential underestimation in the pons tracer uptake associated with partial volume effects would presumably affect the image data in patients and controls similarly, and thus would not be expected to result in differences in BP between groups.

Based largely on preclinical evidence, the pathophysiologic mechanism of PD was hypothesized to involve a fear network centered in the amygdala and its interaction with the hippocampus, medial PFC, and brainstem.[33] This model posited that reduced cortical control (medial PFC), dysregulated contextual fear learning (hippocampus), impaired extinction learning (infralimbic cortex), and hyperactive anxiogenic limbic and paralimbic brain areas (ie, amygdala, hypothalamus, and periaqueductal gray matter[34,35]) contributed to the pathophysiologic mechanism of PD. Compatible with this model, we found abnormalities in BZD receptor binding in PD in the hippocampus/parahippocampal cortex and medial PFC structures such as the DALPFC (Brodmann area 9), frontal polar cortex (Brodmann area 10), anterior cingulate cortex, and infralimbic cortex.[34]

Reduced BZD receptor binding in the medial PFC may reflect downregulation of BZD receptor expression or sensitivity.[36] Such effects may result from repeated stress associated with recurrent panic[37] because preclinical studies show that exposure to acute inescapable stress results in decreased cortical BZD receptor binding.[36,37] In PD subjects, acute panic attacks resulted in pronounced changes in neurosteroid concentrations that were interpreted to indicate decreased GABAergic tone.[38] Moreover, in PD subjects, the basal hypersecretion of the neurosteroids progesterone and its metabolites, pregnenolone and allopregnanolone, which appear to exert anxiolytic effects via positive allosteric modulation of $GABA_A$ receptors,[39] is hypothesized to reflect a compensatory response to blunted $GABA_A$ receptor sensitivity in PD.[40] Finally, reduced brain GABA concentrations were reported in MR spectroscopy studies of PD,[41,42] which also may be associated with altered BZD receptor binding.

The most significant reductions in BZD receptor binding were located in the right DALPFC. This region corresponds to Brodmann area 9, a medial PFC region that shares extensive anatomical connectivity to the periaqueductal gray matter.[34,35] The periaqueductal gray mat-

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ter was implicated in preclinical models of PD[43] because stimulation of the dorsolateral and lateral columns of the periaqueductal gray matter produced fear behaviors and sympathetic autonomic arousal.[44,45] Another medial PFC region where BZD receptor binding was decreased in PD (Table 2) was the anterior cingulate cortex. Hemodynamic activity in the anterior cingulate cortex increased during a variety of anxiety states elicited in healthy or anxiety-disordered subjects,[46] including pentagastrin-induced panic attacks in PD subjects.[47]

The specific association between pure PD (ie, without comorbid MDD; Table 3) and reduced BZD receptor binding in another medial PFC structure, the infralimbic cortex, is noteworthy because projections from this region to the amygdala are implicated in fear extinction learning.[48] Moreover, in rats, pairing conditioned threat cues with brief electrical stimulation of the infralimbic cortex reduces fear.[49] It remains unclear whether reduced BZD-$GABA_A$ receptor binding in this region in PD would be associated with dysregulated extinction learning or might reflect a compensatory mechanism for enhancing neuronal transmission to the amygdala related to fear inhibition.[48] Because exposure-based treatment of PD presumably depends on extinction learning, dysfunction involving the infralimbic cortex may hold clinical relevance in PD.

This is, to our knowledge, the first study to report increased BZD receptor binding in the parahippocampal gyrus, although spatial resolution limitations precluded our ability to definitively resolve this cortex from adjacent hippocampus. This abnormality appeared to be specifically associated with PD, and not with comorbid depression. The parahippocampal gyrus and hippocampus previously were implicated in functional and structural neuroimaging studies of PD. Reiman et al[50] reported an abnormally low left to right ratio of parahippocampal blood flow at rest in PD subjects who were sensitive to lactate-induced panic. De Cristofaro et al[51] found a significant decrease in perfusion in the hippocampus bilaterally in PD subjects. A neuromorphometric MRI study found decreased gray matter density in the parahippocampal cortex of PD subjects vs controls.[52] Increased BZD receptor binding in the parahippocampal region conceivably may reflect a compensatory mechanism in PD. The parahippocampal gyrus and anterior ventral hippocampus (ie, the subiculum) share extensive anatomical projections with the amygdala and medial PFC regions that form the visceromotor network.[34]

The results of the correlational analyses appear paradoxical because they run counter to the group differences. One hypothesis that could reconcile the direction of the group differences and the clinical correlations would hold that changes in BZD receptor binding in PD reflect effective compensatory changes. In this model, higher BZD receptor binding in the hippocampus would result in lower anxiety levels in PD subjects by enhancing the inhibitory tone on limbic activity that facilitates emotional expression.[53] In contrast, lower BZD receptor binding in the DALPFC would be associated with diminished anxiety because reduced inhibitory tone would enhance cortical modulation of anxiety-mediating limbic structures.[48,54,55]

In conclusion, the abnormalities of BZD receptor binding in PD suggest that basal and/or compensatory changes in the GABAergic system are involved in the pathophysiologic mechanism of PD. The reduced BZD binding in the PFC, superior temporal cortex, and parietooccipital cortex were compatible with preclinical evidence that stress downregulates BZD receptor binding, and suggested that this effect may be an important mechanism in the pathogenesis of PD. These data encourage future studies designed to investigate genetic and environmental influences on BZD-$GABA_A$ receptors and interactions among BZD-$GABA_A$, neurosteroids, and monoaminergic and glutamateric receptor systems in PD.

Submitted for Publication: September 12, 2007; final revision received February 13, 2008; accepted March 17, 2008.
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Financial Disclosure: None reported.
Funding/Support: This study was supported by the Intramural Research Program of the National Institutes of Mental Health.

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