

# Decreased Brain GABA<sub>A</sub>-Benzodiazepine Receptor Binding in Panic Disorder

## Preliminary Results From a Quantitative PET Study

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**Background:** Positron emission tomography (PET) allows the measurement of benzodiazepine- $\gamma$ -aminobutyric acid<sub>A</sub> (GABA<sub>A</sub>) receptor kinetics. We employed flumazenil radiolabeled with carbon 11, a radioligand that labels the benzodiazepine site on the GABA<sub>A</sub> receptor, and fully quantitative, high-sensitivity PET to test the hypothesis that central benzodiazepine site binding is decreased in medication-free patients with panic disorder.

**Methods:** We compared 7 patients with panic disorder who had been off medication for at least 6 months and who had never abused alcohol with 8 healthy controls. The resulting parametric voxel-by-voxel maps were analyzed by voxel-based and region of interest-based methods using both parametric and nonparametric statistics.

**Results:** The major finding was that there is a global reduction in benzodiazepine site binding throughout the brain in patients with panic disorder compared with controls. There were sex differences in the 2 samples, but a separate analysis excluding women led to the same conclusions. In addition, the loci with the largest regional decrease in binding (right orbitofrontal cortex and right insula) were areas thought to be essential in the central mediation of anxiety.

**Conclusion:** These results must be considered preliminary but are congruous with previous clinical psychopharmacologic evidence of involvement of the benzodiazepine-GABA<sub>A</sub> receptor and demonstrate that decreased flumazenil binding at this site may underlie panic disorder.

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**P**ANIC DISORDER is an anxiety disorder characterized by spontaneous paroxysms of severe fear<sup>1</sup>; it has a prevalence of 1% to 2%<sup>2,3</sup> and results in considerable morbidity<sup>4</sup> and increased mortality.<sup>5</sup>

Pharmacologic and metabolic probes have revealed that in panic disorder there are alterations of serotonergic,<sup>6-8</sup> noradrenergic,<sup>9-12</sup> GABAergic,<sup>13,14</sup> and brain cholecystokinin<sup>15,16</sup> function and that there is corticosteroid dysregulation<sup>17</sup> as well as increased sensitivity to the anxiety provoking effects of carbon dioxide<sup>18,19</sup> and lactate.<sup>20,21</sup> Thus, panic disorder may be due to the excessive and inappropriate activation of evolutionary valuable alarm systems, which could be the result of a failure of inhibition secondary to benzodiazepine- $\gamma$ -aminobutyric acid (GABA) dysfunction. We postulate that this is central to panic disorder.

The evidence for GABAergic involvement in panic disorder is that blocking GABA<sub>A</sub> receptors with antagonists leads to severe anxiety in man and in animals,<sup>22,23</sup>

whereas increasing GABA function with agonists reduces anxiety.<sup>24,25</sup> Additionally, modulating GABA effects with benzodiazepine site ligands results in anxiety modulation, so that agonists (eg, alprazolam) are panicolytic, while inverse agonists are panicogenic.<sup>26,27</sup> Moreover, ontogenetic or phylogenetic alterations in receptor numbers or subtypes are associated with increased anxiety-like behaviors in animals.<sup>28-30</sup> Finally, patients with panic disorder are less sensitive to benzodiazepines on a number of psychophysiological measures, such as saccadic eye movements to target and suppression of the norepinephrine appearance rate.<sup>13,31</sup>

These findings and the discovery of putative endogenous inverse agonists in man (diazepam-binding inhibitor<sup>32</sup> and tribulin<sup>33</sup>) led to theories of panic being precipitated by the pathologic production of a putative endogenous inverse agonist. However, Nutt and colleagues<sup>14</sup> discovered that flumazenil, a benzodiazepine site antagonist that has neutral anxiety effects in control subjects, provokes panic attacks in patients with panic disorder, dis-

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## PATIENTS AND METHODS

### PATIENTS

Inclusion criteria for patients and volunteers were age 21 to 65 years, no medication for more than 6 months, never having had a prescription for benzodiazepines for anxiety, and no or moderate alcohol use. Patients and volunteers with excessive use of alcohol (>28 U/wk for men and >21 U/wk for women [1 U = 8 g]) and current (or previous) regular use of benzodiazepines were excluded, as they might have produced a downregulation of the BZ site that would have contaminated the data.<sup>28,34</sup> Patients had to fulfill the criteria for a *DSM-IV* diagnosis of panic disorder and had to have no concurrent axis I or III diagnoses. Controls had to be men or postmenopausal women (regulatory requirement) with no current or past axis I diagnosis and no current axis III diagnosis. Patients and volunteers had an extensive clinical interview by an experienced psychiatrist (A.L.M.); semistructured interviews done by specialist physicians have previously been shown to have a reasonable concordance with structured interviews, such as the Structured Clinical Interview for *DSM-III-R*.<sup>35</sup> Clinical information, including past prescriptions, was also obtained from primary care physicians, who hold personal medical history records throughout life. Patients were recruited from the Bristol Psychopharmacology Clinic and from a voluntary organization providing support for patients with anxiety disorders. Volunteers were recruited by advertisement. This study was approved by the Administration of Radioactive Substances Advisory Committee of the Department of Health and by the ethics committees of the Hammersmith Hospitals and Royal Postgraduate Medical School. Written informed consent was obtained from each patient and healthy volunteer.

The study sample comprised 8 healthy male volunteers and 7 patients (4 men, 3 women). The mean (SD) ages

were 38.9 (9.0) years for the controls and 38.1 (18.4) years for the patients (Mann-Whitney  $z = 0.64$ , not significant). Mean alcohol consumption was 14.5 (9.3) U/wk for controls and 8.7 (6.2) U/wk for patients (Mann-Whitney  $z = 1.16$ , not significant). The state anxiety score at the time of scanning, as measured by the Spielberger State Anxiety Inventory,<sup>36</sup> was 28.6 (5.3) for controls and 39.9 (12.9) for patients (Mann-Whitney  $z = 1.96$ ,  $P = .05$ ). Clinical evaluation determined the presence of a previous comorbid psychiatric disorder in 1 patient (major depressive disorder). One patient had taken temazepam for 1 week for insomnia, and 3 controls had occasionally used benzodiazepines for jet lag or insomnia. Three patients had experimented with illicit drugs when younger; in all cases they had not used any illicit drugs for at least 6 months. All participants had negative findings on urine screens for benzodiazepines, alcohol, and drugs of abuse at the time of the scan. Patients were further clinically characterized using the Agoraphobic Cognitions Questionnaire,<sup>37</sup> the Spielberger Trait Anxiety Inventory and Spielberger State Anxiety Inventory,<sup>36</sup> and the Marks and Sheehan Phobia Scale.<sup>38</sup> Further clinical details are summarized in **Table 1**. Details from volunteers are summarized in **Table 2**.

### PET FACILITIES AND PROCEDURES

We measured brain GABA<sub>A</sub>-benzodiazepine receptors in panic disorder using the labeled antagonist [<sup>11</sup>C]flumazenil and fully quantitative PET. Scans were obtained using a model 953B PET scanner (CTI Inc, Knoxville, Tenn) with the collimating septa retracted (full-width half maximum, 8 mm transaxial and 4 mm axial). This "3-dimensional" technique increases the sensitivity of the scanner up to fivefold.<sup>39</sup> However, careful calibration is needed with each scan, as the sensitivity of the crystals to photons in different energy windows<sup>40</sup> needed for measured scatter correction varies with temperature changes. Therefore, for all these studies, we performed a scan with a germanium 68 phantom to calibrate scanner

**Table 1. Clinical Indices for Patients\***

Age, y/ Sex	SSAI Score†	STAI Score†	Time Since First Episode, y	Treatment‡	Current Episode, mo	Alcohol Intake, U/wk§	Family History	ACQ Score	MS Score¶	Volume of Distribution#
65/M	21	37	20	No	6	2	No	16	24	3.15
57/M	47	44	4	Yes	2	12	Yes	40	36	2.76
22/M	63	54	3	No	36	20	Yes	32	51	3.17
49/M	39	60	2.5	Yes	30	8	No	36	24	3.10
29/F	31	38	19	No	6	10	Yes	24	33	3.60
24/F	35	60	1.5	Yes	18	7	Yes	49	27	3.13
21/F	43	55	2	No	24	2	No	37	54	3.86

\*SSAI indicates Spielberger State Anxiety Inventory; STAI, Spielberger Trait Anxiety Inventory; ACQ, Agoraphobic Cognitions Questionnaire; and MS, Marks and Sheehan Phobia Scale. For each of these instruments, the higher the score, the greater the morbidity.

†Range, 20 to 80.

‡Whether patients had ever received pharmacotherapy for panic disorder.

§One unit equals 8 g.

||Range, 14 to 70.

¶Questions 2 to 13 (range, 0-120).

#For the whole, spatially normalized, and smoothed scan.

proving the putative benzodiazepine receptor inverse agonist theory. Two possible explanations may account for this finding: The first is that changed binding/function at the benzodiazepine-GABA<sub>A</sub> receptor alters the effects of

flumazenil so that it behaves like an inverse agonist. The second is that flumazenil blocks a putative endogenous agonist that is present in a compensatory function but is insufficient to prevent the emergence of panic attacks.

counts, and we also obtained a measured attenuation scan using rotating germanium 68 rods with the patient in situ. Patients were positioned in the scanner parallel to the canthomeatal line using laser lines whose positions were known with respect to the camera. The field of view of the scanner is 11 cm in the z direction; hence, our data set consists mainly of data from the middle and lower parts of the brain; for the whole group, this resulted in available data from 4 cm below to 5 cm above the anterior commissure–posterior commissure plane. For each session, 340 MBq ( $9.2 \times 10^{-9}$  Ci) of [ $^{11}\text{C}$ ]flumazenil was injected intravenously in the left antecubital vein, and data were collected for 90 minutes. Each subject had a radial artery cannula to allow continuous counting of blood radioactivity concentrations with a bismuth germanate<sup>41</sup> counter (an arterial input function is needed to quantify brain concentrations of the ligand with respect to blood). Discrete samples were taken at 2.5, 10.5, 20.5, 35.5, 50.5, and 65.5 minutes for calibration of the count data over a well counter and to determine the plasma counts fraction, and samples were taken at 3, 4, 5, 6, 10, 20, 35, and 60 minutes to measure concentrations of [ $^{11}\text{C}$ ]flumazenil and its metabolites. The metabolite concentration was subtracted from the total, resulting in a metabolite-corrected plasma input function. Twenty-four time-dynamic frames were acquired (3 of 1 minute, 19 of 3 minutes, and 2 of 15 minutes). The brain volume of distribution of this tracer was assessed using voxel-by-voxel spectral analysis<sup>42</sup> of PET images. This method allows measures of receptor binding to be clearly separated from possible confounding factors, such as blood flow (delivery).

[ $^{11}\text{C}$ ]Flumazenil is a radiolabeled benzodiazepine site PET ligand with very favorable characteristics for studying benzodiazepine-GABA<sub>A</sub> (BZ) receptor kinetics in man in vivo, as it crosses the blood-brain barrier easily, does not bind to other receptors, has high affinity, and is not metabolized in the brain, while its plasma metabolites are highly polar and therefore do not access brain tissue.<sup>43</sup> Brain volume of distribution is a receptor kinetics measure that is proportional

to the maximum number of available binding sites divided by the dissociation constant (binding potential); it is the sum of the signal from all 3 tissue compartments (free plus non-specific binding plus specific binding). This measure is independent of blood flow and can be obtained using only 1 PET scan,<sup>42</sup> an important consideration when studying patients with panic disorder, who may find it difficult to endure more than 1 scanning session. Prior to spectral analysis, the various dynamic frames were all realigned using rigid body transformations, as in the realignment procedure in statistical parametric mapping.<sup>44</sup> This was necessary to control for possible patient movement, although subjects were regularly checked for movement within the scanner by visualizing external markers drawn over the skull's bony landmarks and aligned with laser beams within the scanner inlet. [ $^{11}\text{C}$ ]Flumazenil is an ideal ligand for realignment, as it has a wide and approximately uniform cortical distribution.

## STATISTICAL ANALYSIS

Two separate methods were used to analyze the data (between-subjects test), as there is no agreed optimal method. Voxel-by-voxel analysis avoids the sampling bias associated with region of interest (ROI) selection but has only been validated for use with parametric analyses. Therefore, we also employed ROI analysis and nonparametric methods, as volume of distribution values may not be normally distributed.

Hence, for parametric voxel-by-voxel analysis, the parametric images were put in stereotactic space, smoothed with a 15-mm gaussian filter, and compared using statistical parametric mapping (SPM95)<sup>45-47</sup> without global normalization, as the scans were fully quantitative.

For nonparametric analysis, ROIs were drawn using an automated stereotactic atlas of regions positioned on the spatially normalized volume of distribution images, as in previous studies.<sup>48</sup> Comparisons were then carried out using the Wilcoxon–Mann-Whitney test.

The purpose of this study is to investigate whether patients with panic disorder have reduced brain benzodiazepine-GABA<sub>A</sub> binding in vivo that would explain the existing pharmacologic challenge and clinical data. To this end, we employed fully quantitative positron emission tomography (PET) using flumazenil radiolabeled with carbon 11 to compare the brain volume of distribution of this ligand between patients and controls. The volume of distribution is the ratio of plasma to brain ligand distribution at equilibrium. In accordance with previous work, we hypothesized that decreased binding would be found in limbic and paralimbic structures as well as in some parts of the complex brain network subserving the generation of voluntary saccades to target.<sup>13</sup>

## RESULTS

A significant, global, contiguous decrease in volume of distribution was detected in the whole brain according to spatial extent criteria (41627 of 55839 total voxels;  $P < .001$  for fully corrected statistical parametric mapping extent criteria), with peak decreases in the right orbitofrontal cortex, right insula, right lingual gyrus, left fusiform gyrus, right superior temporal gyrus, left middle temporal gyrus, right

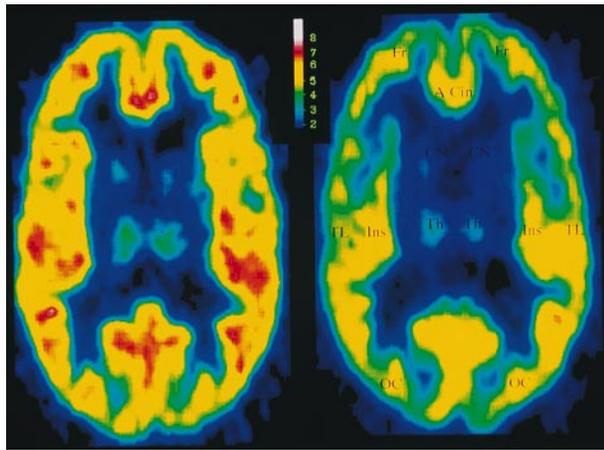
**Table 2. Characteristics of Healthy Volunteers\***

Age, y/Sex	Alcohol Intake, U/wk	SSAI Score	Volume of Distribution
34/M	25	32	3.67
43/M	8	37	3.29
43/M	12	27	3.94
36/M	1	33	4.74
33/M	7	28	3.89
29/M	20	24	3.52
35/M	15	28	3.42
58/M	28	20	3.24

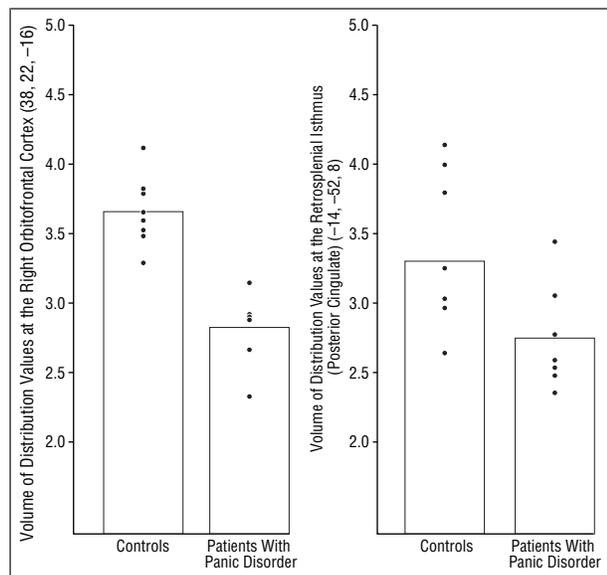
\*SSAI indicates Spielberger State Anxiety Inventory.

middle temporal gyrus, left dorsolateral frontal cortex, left anterior medial frontal cortex, and left frontal pole. Benzodiazepine volume of distribution maps of a middle brain slice ( $z = +12$  mm, ie, 12 mm above the anterior commissure–posterior commissure plane) are shown for the median volume of distribution value in each group (**Figure 1**).

The mean volume of distribution values for single brain voxels in the panic disorder group are between 76%



**Figure 1.** Middle brain (12 mm above the anterior commissure–posterior commissure plane) median volume of distribution map for controls (left) and patients with panic disorder (right). Note the generalized decrease in volume of distribution affecting the thalami and the heads of the caudate nucleus. FC indicates frontal cortex; CN, caudate nucleus; Th, thalamus; Ins, insula; TC, temporal cortex; and OC, occipital cortex.



**Figure 2.** Volume of distribution values for the panic disorder and control groups at the voxels with the greatest (left,  $z = 4.22$ ) and least (right,  $z = 1.96$ ) significant difference within the gray matter.

and 82% of the mean values in the control group, as shown for the voxels with the maximal (right orbitofrontal cortex) and minimal (left retrosplenial isthmus–posterior cingulate) differences within the spatial extent significance volume (**Figure 2**).

Comparison of the pooled regional averages for the 26 regions for which we have ROI values using the nonparametric Wilcoxon–Mann–Whitney test also shows significant global reduction in binding ( $z = 6.99$ ;  $P < .001$ ). Individual regional  $z$  scores using a Wilcoxon–Mann–Whitney test are shown (**Table 3**). Nonparametric comparison of male participants (8 controls vs 4 patients with panic disorder) shows similar (if not greater) differences, and these data are also presented in Table 3. This comparison is important, as it argues against the notion that the observed differences are due to the sex differences between the samples.

**Table 3. Volume of Distribution Values Using [<sup>11</sup>C]Flumazenil by Region of Interest**

Site	Mean (SD) Volume of Distribution		Nonparametric Wilcoxon–Mann–Whitney <i>U</i> Test	
	Controls	Patients With Panic Disorder	All Subjects	Men Only
Cerebellum				
L	4.26 (0.55)	3.89 (0.39)	-1.16	-2.72
R	4.17 (0.44)	3.74 (0.43)	-1.62	-2.04
Thalamus				
L	3.01 (0.14)	2.43 (0.44)	-3.24	-2.21
R	3.31 (0.48)	2.80 (0.42)	-2.26	-1.87
Basal ganglia				
L	2.94 (0.33)	2.42 (0.19)	-2.78	-2.72
R	2.47 (0.26)	2.10 (0.27)	-2.20	-2.55
Amygdala/hippocampus				
L	4.46 (0.35)	3.77 (0.51)	-2.55	-1.87
R	4.43 (0.55)	3.75 (0.42)	-2.43	-1.19
Postmedial temporal				
L	5.63 (0.48)	4.97 (0.53)	-1.74	-2.72
R	5.86 (0.58)	5.14 (0.48)	-0.93	-2.72
Lateral temporal				
L	5.00 (0.53)	4.34 (0.47)	-2.20	-2.72
R	5.55 (0.61)	4.66 (0.50)	-2.66	-2.72
Medial occipital				
L	5.11 (0.55)	4.47 (0.58)	-2.72	-2.55
R	6.17 (0.89)	5.35 (0.51)	-2.96	-2.55
Lateral occipital				
L	5.39 (0.60)	4.69 (0.42)	-2.19	-2.72
R	5.13 (0.76)	4.53 (0.53)	-2.31	-2.55
Medial frontal				
L	4.66 (0.61)	3.95 (0.47)	-2.43	-2.72
R	5.38 (0.71)	4.43 (0.55)	-2.08	-2.72
Anterior and dorsolateral prefrontal				
L	4.75 (0.45)	4.09 (0.57)	-2.78	-2.72
R	4.74 (0.69)	4.01 (0.54)	-2.55	-2.72
Orbitofrontal				
L	3.85 (0.51)	3.47 (0.43)	-2.66	-2.72
R	4.68 (0.52)	3.96 (0.56)	-2.43	-2.72
Insula				
L	5.21 (0.42)	4.44 (0.59)	-1.62	-2.72
R	4.93 (0.59)	4.03 (0.47)	-2.78	-2.72
Anterior cingulate				
L	5.78 (0.63)	4.82 (0.54)	-2.90	-2.72
Pons				
L	1.73 (0.16)	1.59 (0.24)	-1.16	-2.72

Nobody experienced a panic attack either during the scanning session or during the preparation period.

#### COMMENT

We have demonstrated significantly decreased benzodiazepine receptor binding. This is consistent with the idea that panic disorder may be due to defective brain inhibition that leads to or allows paroxysmal elevations in anxiety during panic attacks. The fact that no one experienced a panic attack during a scan suggests that the observed changes are due either to baseline changes in receptor binding (a decrease in the maximum number of binding sites or an in-

crease in the dissociation constant) or to increased occupancy of the receptor by an endogenous ligand, perhaps in the context of the increased anxiety tone. The peak decreases in benzodiazepine binding were in anatomical areas thought to be involved in the experience of anxiety in man (eg, the orbitofrontal cortex and insula).<sup>49-52</sup> If panic disorder is caused by a dysfunctional alarm system,<sup>53</sup> global alterations in brain chemistry might be expected, because an effective extreme danger reaction has to involve the whole brain, and interruption of other ongoing behaviors is potentially vital for the survival of the whole organism.

Previously, other groups have attempted to examine the theory of reduced binding at the benzodiazepine-GABA<sub>A</sub> receptor by using iomazenil single photon-emission computed tomography.<sup>54-56</sup> Some of these studies have methodologic limitations (inappropriate control groups, relative quantitation only, presence of medication, and, most important, too short an interval between injection and scanning to separate the effects of delivery from binding) that result in considerable difficulty in interpreting the data. In addition, none of these studies measured plasma concentrations of iomazenil and therefore cannot produce fully quantitative data. Our study is itself limited by the small numbers studied and the imbalance in the sex ratio between the groups. The sex ratio, however, is unlikely to explain the results, as a separate comparison excluding women led to the same conclusions.

Small numbers and an imbalance in the sex mix reflect the difficulty in recruiting these patients for imaging studies. In the present study, it took more than 2 years to accrue this number of well-screened patients with the necessary characteristics and whose scans were fully quantitative.

There are 3 main types of mechanisms that could account for the widespread reduction in binding that are not mutually exclusive. First, an alteration of the subunit composition of the benzodiazepine-GABA<sub>A</sub> complex could indicate an a priori differential expression of GABA<sub>A</sub>-benzodiazepine receptors in patients with panic disorder. For instance, increased expression of  $\alpha_6$  subunits, to which [<sup>11</sup>C]flumazenil does not bind, would produce the current findings. Polymorphisms of benzodiazepine-GABA<sub>A</sub> receptors might result in distinct receptor variants being more prevalent in panic disorder<sup>57</sup>; rat strains that are more anxious have been observed to have decreased benzodiazepine binding<sup>30</sup> and decreased anticonflict effects from the same doses of benzodiazepine agonist.<sup>58</sup> Decreased binding secondary to subunit composition modification could also be due to environmentally induced modification in receptor configuration<sup>59</sup> or endocytic loop phosphorylation.<sup>60</sup>

Second, the presence of putative endogenous benzodiazepine ligands, increased GABA concentration,<sup>61</sup> or neurosteroid inverse agonists<sup>62</sup> can induce reduced benzodiazepine binding and may mediate the corticosteroid-dependent reduced binding observed with long-term stress in mice.<sup>63-65</sup> Finally, changes in serotonergic<sup>66,67</sup> or noradrenergic<sup>68,69</sup> tone are known to affect benzodiazepine receptor binding. Other explanations are possible but less plausible: a global decrease due to gray-matter atrophy is very unlikely, and magnetic resonance imaging studies of panic disorder have only found minimal changes, mostly in the temporal lobe.<sup>70</sup> Changes in nonspecific binding of flumazenil in the brain, which would also decrease the volume of

distribution, would not be of this magnitude, as nonspecific binding accounts for only about 10% of the total volume of distribution.<sup>71</sup> Decreased binding due to occupancy by an anxiogenic endogenous inverse agonist is also unlikely to explain these findings because the pure antagonist flumazenil would be anxiolytic and not panicogenic, as previously demonstrated.<sup>14</sup>

Because of the complex system interactions in the brain, it is likely that a number of neurotransmitters are involved, and other receptors could be altered in panic disorder. Thus, future investigation should include the mapping of monoaminergic receptor density in patients with panic disorder as well as a comparison of benzodiazepine-GABA<sub>A</sub> receptors using subtype specific ligands. In addition, the technology is now mature for the measurement of the effects of stress on benzodiazepine binding in man in vivo.

In conclusion, this study has shown probable decreased binding at the brain GABA<sub>A</sub>-benzodiazepine site in panic disorder, strengthening the case that abnormalities in basal or adaptive inhibitory neuromodulation are of pathologic significance in this condition.

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