
SPECT [I-123]Iomazenil Measurement of the Benzodiazepine Receptor in Panic Disorder

J. Douglas Bremner, Robert B. Innis, Thomas White, Masahiro Fujita, David Silbersweig, Andrew W. Goddard, Lawrence Staib, Emily Stern, Angela Cappiello, Scott Woods, Ronald Baldwin, and Dennis S. Charney

Background: Alterations in benzodiazepine receptor function have long been hypothesized to play a role in anxiety. Animal models of anxiety involving exposure to chronic stress have shown a specific decrease in benzodiazepine receptor binding in frontal cortex and hippocampus. The purpose of this study was to examine benzodiazepine receptor binding patients with panic disorder and comparison subjects.

Methods: A quantitative measure related to benzodiazepine receptor binding (Distribution Volume (DV)) was obtained with single photon emission computed tomography (SPECT) imaging of [¹²³I]iomazenil and measurement of radioligand concentration in plasma in patients with panic disorder and healthy controls. DV image data were analyzed using statistical parametric mapping (spm96).

Results: A decrease in measures of benzodiazepine receptor binding (DV) was found in left hippocampus and precuneus in panic disorder patients relative to controls. Panic disorder patients who had a panic attack compared to patients who did not have a panic attack at the time of the scan had a decrease in benzodiazepine receptor binding in prefrontal cortex.

Conclusions: Findings of a decrease in left hippocampal and precuneus benzodiazepine receptor binding may be related to alterations in benzodiazepine receptor binding, or other factors including changes in GABAergic transmission or possible endogenous benzodiazepine compounds. Benzodiazepine receptor function in prefrontal cortex appears to be involved in changes in state-related panic anxiety. *Biol Psychiatry* 2000;47:96–106 © 2000 Society of Biological Psychiatry

Key Words: Panic disorder, SPECT, neuroimaging, benzodiazepines

Introduction

Changes in benzodiazepine receptor function have been hypothesized to be involved in the pathophysiology of the anxiety disorders. (Guidotti et al 1990). The animal model of inescapable stress has been used as a model for human anxiety disorders and for the development of medications for the treatment of anxiety (reviewed in Charney et al 1996). Animals repeatedly exposed to inescapable stress developed a number of behaviors, such as increased fearfulness, increased defecation, and avoidance of novel situations like an open field, that were noted to parallel symptoms of pathological anxiety in humans. These animals also developed a 20–30% decrease in benzodiazepine receptor binding in frontal cortex (Lippa et al 1987; Weizman et al 1989), cerebral cortex (Drugan et al 1986, 1989; Medina et al 1983a, 1983b; Weizman et al 1990), and hippocampus (Drugan et al 1986, 1989; Havoundjian et al 1986; Medina et al 1983a, 1983b; Skerritt et al 1981; Weizman et al 1989, 1990). Exposure to stress had no effects on benzodiazepine receptor binding in pons, striatum, thalamus, cerebellum, midbrain, or occipital cortex (Braestrup et al 1979; Drugan et al 1986; Havoundjian et al 1986; Lefur et al 1979; Lippa et al 1987; Medina et al 1983a, 1983b; Miller et al 1987; Skerritt et al 1981; Weizman et al 1990). A decrease in benzodiazepine receptor binding (B_{max}) was also demonstrated in the Maudsley genetically fearful strain of rat in comparison to nonfearful rats in several brain structures including the hippocampus (Robertson et al 1978). These studies suggest that decreased benzodiazepine receptor binding in frontal cortex and hippocampus may play a role in the pathophysiology of anxiety disorders.

Studies in patients with anxiety disorders and exposure to stress have also investigated correlates of the benzodiazepine receptor (Rocca et al 1992; Weizman et al 1987, 1994). Panic disorder patients were less sensitive than controls to diazepam using saccadic eye movement velocity as a dependent measure, suggesting a functional subsensitivity of the GABA-benzodiazepine supramolecular complex in brain stem regions controlling saccadic eye

From the Departments of Psychiatry (JDB, RBI, AWG, AC, SW, DSC) and Diagnostic Radiology (JDB, MF, LS, RB), Yale University School of Medicine, New Haven, Connecticut; VA Connecticut Healthcare System (JDB, RBI, MF, AC, RB, DSC) and National Center for PTSD-West Haven (JDB, RBI, DSC), West Haven, Connecticut; and Department of Psychiatry, Cornell University School of Medicine, New York, New York (TW, DS, ES).

Address reprint requests to J. Douglas Bremner, MD, Yale Psychiatric Institute, P.O. Box 208038, Yale Station, New Haven, CT 06520.

Received November 10, 1998; revised June 30, 1999; accepted July 17, 1999.

movements (Roy-Byrne et al 1990). Panic patients also had a diminished sensitivity to suppression of plasma norepinephrine and epinephrine and suppression of pulse rate following administration of diazepam in comparison to controls (Roy-Byrne et al 1989). Administration of the benzodiazepine receptor antagonist flumazenil to patients with panic disorder resulted in an increase in panic attacks and subjective anxiety in comparison to controls (Nutt et al 1990; Woods et al 1991). The benzodiazepine receptor inverse agonist, FG7142, induced severe anxiety resembling panic attacks and biological characteristics of anxiety in healthy subjects (Dorow et al 1983). Perhaps the most convincing piece of evidence linking benzodiazepine receptor function to the anxiety disorders is the efficacy of the benzodiazepines in their treatment.

Iomazenil binds with high affinity to the benzodiazepine receptor and can be used for imaging of the benzodiazepine receptor with either ^{11}C and PET or ^{123}I and SPECT. SPECT has been used to show a decrease in [^{123}I]iomazenil uptake in frontal (Kascka et al 1995; Kuikka et al 1995; Shlegel et al 1994), temporal (Kascka et al 1995; Shlegel et al 1994) and occipital (Shlegel et al 1994) cortex in panic disorder patients relative to comparison subjects. These studies were limited by the use of non-quantitative methods for estimation of benzodiazepine receptor binding (Kascka et al 1995; Kuikka et al 1995; Shlegel et al 1994), absence of medication free subjects (Kascka et al 1995; Shlegel et al 1994), psychiatric comorbidity within panic disorder subjects (Kascka et al 1995), or the use of diseased patients for comparison groups (Kascka et al 1995; Shlegel et al 1994). One recent study used positron emission tomography (PET) and [^{11}C]flumazenil in the measurement of benzodiazepine receptor binding and found global decreases in panic disorder patients versus controls, with the greatest magnitude in right orbitofrontal cortex and insula (Malizia et al 1998).

Reliable and valid methods were developed for quantitation of benzodiazepine receptor binding using (SPECT) [^{123}I]iomazenil with both kinetic and equilibrium techniques (Abi-Dargham et al 1994, 1995; Innis et al 1991). In our hands we have shown good agreement between quantitation of benzodiazepine receptor binding with PET and SPECT in the same healthy human subjects using kinetic techniques (Bremner et al, in press). The purpose of this study was to measure benzodiazepine receptor binding using SPECT and [^{123}I]iomazenil in patients with panic disorder and matched normal comparison subjects. Based on preclinical studies reviewed above, we hypothesized a decrease in benzodiazepine receptor binding in frontal cortex and hippocampus, but not in other areas (pons, striatum, thalamus, cerebellum, or midbrain) in patients with panic disorder relative to normal comparison subjects.

Methods and Materials

The patient group was composed of 13 individuals with panic disorder between the ages of 18 and 65 who gave informed consent for participation. Patients were recruited by newspaper advertisement or through clinician referral, met DSMIV criteria for panic disorder based on the Structured Clinical Interview for DSMIV (SCID) (Spitzer et al 1987) or the Adult Diagnostic Interview Schedule (ADIS), and were presenting for evaluation and treatment in a specialized anxiety disorder clinic. Comparison subjects ($n = 16$) were recruited by newspaper advertisement and were selected to be similar to the patients in age. There was no difference in age between panic disorder patients and comparison subjects (43 [12 SD] vs. 45 [10 SD]). There were 12 white and 1 black patients; all of the comparison subjects were white. There were 9 female and 4 male panic disorder patients; there were 8 females and 8 males in the comparison group. All subjects were right-handed. Comparison subjects were screened with the SCID for Non-Patients, and comparison subjects with a history of psychiatric disorder or alcohol/substance abuse or dependence were excluded. Patients and comparison subjects were free of all medications for at least 4 weeks prior to the study. Patients and comparison subjects were free of benzodiazepines or other substances for at least 6 weeks prior to the study as confirmed by urine toxicology at screening and at the time of the scan. Patients and comparison subjects were excluded with a history of current alcohol or drug abuse or dependence, meningitis, traumatic brain injury, loss of consciousness of greater than 10 min, other neurological disorder, or schizophrenia based on the SCID interview. Patients and comparison subjects were also excluded who had a history of foreign bodies that would preclude MRI scanning.

Patients were evaluated with the SCID for comorbid psychiatric diagnoses. Twelve out of 13 (92%) patients met criteria for current and lifetime panic disorder with agoraphobia, and 1 out of 13 (8%) met criteria for current and lifetime panic disorder without agoraphobia. Two out of 13 (15%) patients met criteria for current and lifetime simple phobia, and 1 out of 13 (8%) for current and lifetime social phobia. One out of 13 (8%) patients met criteria for current bulimia, and 2 out of 13 (15%) for lifetime bulimia. None of the patients met criteria for current or lifetime generalized anxiety disorder, obsessive-compulsive disorder, posttraumatic stress disorder, schizophrenia, bipolar disorder, major depression, dysthymia, somatization disorder, somatic pain disorder, undifferentiated somatization disorder, or hypochondriasis. Two patients (13%) met criteria for past alcohol dependence, 2 (15%) for past polydrug dependence, and 1 (8%) for past marijuana abuse. None of the controls had a history of psychiatric disorder.

Patients and comparison subjects were also evaluated at the time of the scan for current panic attack symptoms with the Panic Attack Symptom Scale (PASS), a state measure of current panic symptom severity. A score of greater than 8 on the PASS is consistent with a current panic attack as defined by DSMIV (i.e., greater than 4 panic symptoms rated as moderate in severity or greater). Patients were evaluated for general anxiety symptom level at the time of the scan with the Hamilton Anxiety Scale (HAMA) (Hamilton 1959), and the Clinician Rated Anxiety

Scale (CRAS), state measures of current anxiety level. Panic patients had significantly higher levels of panic symptomatology as measured by the PASS (8.1 [6.7 SD] vs. 0.4 [1.0 SD]) ($t = 4.1$; $df = 27$; $p = .001$) and anxiety as measured by the HAMA (14.5 [8.7 SD] vs. 1.9 [1.7 SD]) ($t = 5.1$; $df = 27$; $p < .001$) and the CRAS (27.5 [12.3 SD] vs. 1.2 [1.2 SD]) ($t = 8.5$; $df = 27$; $p < .001$).

All subjects underwent a single SPECT [^{123}I]iomazenil scan for measurement of benzodiazepine receptor binding using methods previously described (Abi-Dargham et al 1994, 1995). Throughout this paper the term benzodiazepine receptor binding is used for economy of expression to signify binding of [^{123}I]iomazenil to the receptor. Changes in this parameter may be reflective of multiple causes, including changes in endogenous benzodiazepine ligand (the existence of which is controversial), changes in GABA transmission, or changes in receptor affinity or number. Toxicology screen was obtained on the day of the scan to rule out benzodiazepine or other substance usage. Six mCi [^{123}I]iomazenil was administered as a bolus followed by a continuous infusion of radiotracer over 6 hours (ratio of bolus/infusion rate = 3.8 mCi administered as a bolus for each 1 mCi administered per hour as a constant infusion). Starting at 6 hours after administration of the bolus, subjects underwent SPECT scanning at equilibrium of radioligand uptake into the brain, as determined by no change in brain tissue concentration over 3 consecutive scans. At equilibrium (as determined by this method), rate of transfer of radioligand (i.e., [^{123}I]iomazenil) onto the receptor is assumed to be equal to rate of transfer off of receptor. Subjects were placed in a Ceraspect SPECT camera with head positioned along the cantho-meatal line and immobilized with head and chin straps. Subjects underwent 3 10-min acquisitions with the Cerespect. SPECT images were reconstructed from counts set on the iodine photopeak (159 keV) with a 10% symmetric window using filtered backprojection and a Butterworth filter (cutoff = 1 cm, power factor = 10 mm), with 64 slices, 1.67 mm inter-slice distance, 128×128 matrix, with voxel size of 1.67 mm \times 1.67 mm \times 1.67 mm. Adjacent slices were then summed post acquisition to create 3.34 mm thick slices. Uniform attenuation correction ($\mu = 0.15 \text{ cm}^{-1}$) was performed based on an ellipse placed over the skin surface. Ellipse placement on the skin surface was guided by fiducial markers filled with ^{123}I placed on both sides of the head along the canthomeatal line at the time of the scan. Images were reoriented to the canthomeatal line using the fiducial markers. Resolution determined as the value of full width at half maximum (FWHM) of a reconstructed image of a point source of radioactivity in water placed in the center of the field of view is 1.3 cm. Resolution in water is a close approximation to resolution for brain. Typically for SPECT devices there is a decrease in resolution with increasing distance from the center of the field of view (i.e., resolution is worse for cortical areas than for subcortical areas such as hippocampus). A reconstructed SPECT [^{123}I]iomazenil image is shown in Figure 1. A 12 cm cylindrical fluid filled phantom with a known amount of ^{123}I was scanned for the determination of a calibration factor (0.0017 $\mu\text{Ci}/\text{cpm}$) for conversion of radioactivity in the SPECT image (cpm) into absolute units of radioactivity ($\mu\text{Ci}/\text{mL}$).

Blood was drawn for measurement of total and free (or



Figure 1. SPECT [^{123}I]iomazenil transaxial image in a healthy human subject. Radioligand is primarily distributed in cerebral cortex and other grey matter areas with a 20:1 ratio of grey to white matter distribution, reflecting the widespread distribution of benzodiazepine receptors on neurons in the human brain.

non-protein bound) [^{123}I]iomazenil parent compound in plasma using methods previously described (Zoghbi et al 1992). There was no difference between panic disorder patients and comparison subjects in plasma total parent compound (0.0035 $\mu\text{Ci}/\text{mL}$ [0.0008 SD] vs. 0.0041 $\mu\text{Ci}/\text{mL}$ [0.0027 SD]) or in the fraction of free parent in plasma (f_1) (0.338 [0.030 SD] vs. 0.349 [0.061 SD]).

Magnetic resonance (MR) images were obtained in all subjects for coregistration with SPECT. The head was positioned with the canthomeatal line aligned with the laser light for reproducibility of data acquisition and to create MRI images in the same plane as the SPECT images. MRI scans of 3 mm contiguous slices were obtained with a 1.5 Tesla General Electric Signa device. Axial images were acquired with a spoiled GRASS (gradient recall acquisition in the steady state) sequence with TR = 25 msec, TE = 5 msec, NEX = 2, matrix 256×256 , field of view = 24 cm. Both SPECT and MRI images were transferred via computer network to a Sun Sparc10 Workstation. A surface matching algorithm running under ANALYZE was used for coregistration of SPECT and MR images (Jiang et al 1992).

The three SPECT scans were realigned to the first scan in the series and summed into a single image. SPECT scans were then converted from units of cpm to $\mu\text{Ci}/\text{mL}$ using calibration factors based on phantom studies. Distribution volumes (that are related to neuroreceptor binding) were then calculated from the SPECT and plasma data. In the three compartment model, the first

compartment consists of unmetabolized radioligand (i.e., [123 I]iomazenil) in plasma that is available for uptake into the brain, the second compartment is free radioligand and radioligand that is not bound to receptor, and the third compartment is radioligand specifically bound to the receptor. In the current study, radioligand in plasma and radioligand not bound to receptor was considered to be negligible, considering the 20:1 ratio of specific to nonspecific binding with this radioligand (Sybirska et al 1993). Although the SPECT image includes radioligand non-specifically bound to receptor and free radioligand in brain tissue, as well as ligand in the plasma compartment, the primary contribution to the image is from radioligand specifically bound to receptor. Measurement of unmetabolized radioligand in plasma makes it possible to account for differences in availability of ligand for uptake into the brain, or differences in peripheral metabolism or clearance, that may influence activity in the brain in ways that are unrelated to neuroreceptor binding. Therefore the ratio of the concentration of radioligand "bound" to the receptor (as determined from the SPECT image) to the concentration of unmetabolized radioligand in plasma provides a measure (Distribution Volume [DV]) that is related to neuroreceptor binding. Data was analyzed using both concentrations of radioligand in plasma bound to protein and free in plasma, however similar results were obtained using both methods. Data presented is using radioligand not bound to protein in plasma based on our prior studies showing greater reliability using this as an outcome measure (Abi-Dargham et al 1994).

SPECT images in which values for individual voxels (or picture elements) within the brain corresponded to Distribution Volume (DV) values were then constructed by dividing values for radioactivity in each voxel by the plasma concentration of unmetabolized radioligand determined for that particular subject. These SPECT CV images were stripped of extracranial artifact and coregistered to MRI images. Coregistered MRI images were used to determine normalization parameters for transformation of SPECT images into a common anatomical space. This method takes advantage of the superior delineation of anatomical detail by MRI in comparison to SPECT. Images were then smoothed using a three dimensional Gaussian filter to 16 mm full-width half maximum.

Statistical parametric mapping (spm96) was used to compare measures of regional benzodiazepine receptor binding (DV) in panic disorder versus comparison subjects (Friston et al 1991). Analyses were performed using analysis of covariance with global benzodiazepine receptor binding considered as a confounding covariate. SPM allows a statistical comparison between patients and controls on a voxel-by-voxel basis. With this method there is a statistical value (e.g., *t*-statistic) for the difference between groups assigned to every voxel in the brain. Values for *t*-statistic in individual voxels were transformed into *Z* scores and displayed as an image made up of multiple voxels (or picture elements) corresponding to individual anatomical areas of the brain, demonstrating areas of statistically significant differences between the groups. In order to avoid problems with multiple comparisons, hypothesized areas were identified a priori based on studies in animals (i.e., hippocampus and frontal cortex). Areas of decreased benzodiazepine receptor binding in panic

disorder within these hypothesized regions identified using a threshold of $p < .005$ were noted. This threshold is based on prior studies showing a low level of Type I error using this cutoff as the criteria (Reiman et al 1997). Several specific areas, including pons, striatum, thalamus, midbrain, and cerebellum, not hypothesized to show decreased binding were examined in order to demonstrate specificity. The spm type of analysis provides a comprehensive map of the entire brain and other areas were examined on an exploratory basis. Location of areas of significant difference were identified as the distance from the anterior commissure in mm, with *x*-, *y*-, and *z*-coordinates, using the standard Talairach coordinate space (Talairach and Tournoux 1988). The correlation between panic symptomatology (measured with the PASS) at the time of scan and benzodiazepine receptor binding was also examined using spm96.

MRI scans coregistered to SPECT scans were also used to measure quantitative measures of benzodiazepine receptor binding in the subjects in this study using methods previously described in detail (Bremner et al 1998). Circular regions of interest were placed on the subject's MRI scan resliced to correspond to the subject's SPECT DV benzodiazepine receptor image. Regions were placed on the left and right hippocampus and prefrontal cortex (Brodmann's area 9) as well as whole brain. These regions were then used to measure DV in hippocampus and prefrontal cortex from the SPECT scans. Absolute measures of DV as well as the ratio of regional to whole brain DV were compared between patients and controls using ANOVA. Panic disorder patients who experienced a panic attack at the time of the scan (based on DSMIV criteria of four or more panic symptoms, rated as moderately severe on the PASS) were compared to panic disorder patients without a panic attack at the time of the scan for DV in prefrontal cortex (Brodmann's area 9). Correlations between panic symptoms and benzodiazepine receptor binding were performed using Spearman correlations.

Results

There was a significant decrease in measures of benzodiazepine receptor binding (DV) in one of the two hypothesized areas, left hippocampus, in panic disorder patients relative to comparison subjects. This decrease was identified using both spm type analyses as well as a region of interest placed on the subject's coregistered MRI scan. In the spm analysis of SPECT DV images normalized using the patient's MR scan the area corresponded to Talairach coordinates: $x = 12$, $y = -26$, $z = -4$ (*Z* score = 2.76; $p = .003$) (Figure 2; Table 1). Based on the region of interest analysis using the coregistered MRI scan and non-transformed SPECT DV image, there was a 20% reduction in DV normalized to whole brain DV (23% absolute reduction) in the panic disorder patients relative to controls (.79 [.27 SD] vs. .99 [.19 SD]) ($F = 5.29$; $df = 1,28$; $p < .05$) (Figure 3). There was also a decrease in measures of benzodiazepine receptor binding (DV) in a brain area that was not hypothesized a priori to have decreased binding, the precuneus ($x = 6$, $y = -58$, $z =$

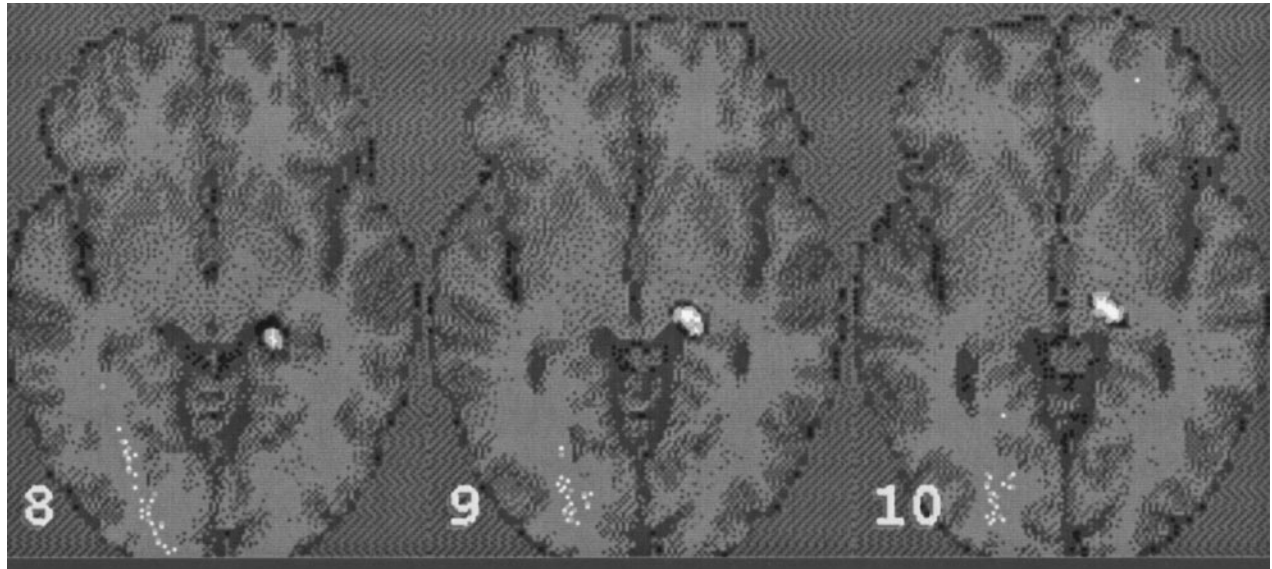


Figure 2. Statistical parametric map overlaid upon an axial MR template image showing areas of significant decrease in benzodiazepine receptor binding (DV) in panic disorder patients relative to controls. Significant decreases were found in left hippocampus (Talairach coordinates: $x = 12, y = -26, z = -4$ [Z score = 2.76; $p = .003$]).

32; Z score = 2.79; $p = .003$) (Table 1). This difference was not confirmed on region of interest analysis. No significant differences were observed in other brain areas not expected to differ between patients and controls (pons, striatum, thalamus, cerebellum, or midbrain). There was no difference in whole brain benzodiazepine receptor binding based on absolute measures of DV between panic disorder patients (29.3 [29.2 SD]) and controls (27.3 [21.5 SD]). These values for DV were similar to our previous reports for both PET and SPECT (Bremner et al 1999, in press).

Several regions showed increased values for DV in panic disorder patients relative to controls based on the spm analysis, with the most significant difference in right caudate (Table 2). There was no a priori hypothesis of increased benzodiazepine receptor binding in panic disorder.

A significant correlation was found between decreased values for DV in one of the hypothesized areas, prefrontal

cortex, and panic attack symptomatology as measured by the PASS in the panic disorder patients. Areas of frontal cortex identified in the spm analysis included superior and middle frontal gyri, or Brodmann's areas 8, 9, and 10 (Table 3; Figure 4). This analysis controlled for differences in global benzodiazepine receptor binding, suggesting specificity to the regions outlined in Table 3. A comparison of benzodiazepine binding (DV) based on a region of interest placed over Brodmann's area 9 between panic disorder patients who did and did not have a panic attack at the time of the scan showed a marked reduction in benzodiazepine receptor binding in the panicking relative to the nonpanicking patients (Figure 5). The lack of difference between the panic group as a whole and controls in this area suggests that this is a state-related

Table 1. Areas of Decreased Benzodiazepine Receptor Binding (DV) in Panic Disorder Patients ($n = 13$) Relative to Comparison Subjects ($n = 16$)

Z score	p-value	Talairach coordinates			Brain region
		x	y	z	
2.79	0.003	6	-58	32	Precuneus
2.76	0.003	12	-26	-4	L. hippocampus
2.76	0.003	16	-32	-8	
2.53	0.006	26	-44	-16	

Regions in bold indicate area of greatest difference with a contiguous cluster of significant voxels.

Table 2. Areas of Increased Benzodiazepine Receptor Binding (DV) in Panic Disorder Patients ($n = 13$) Relative to Comparison Subjects ($n = 16$)

Z score	p-value	Talairach coordinates			Brain region
		x	y	z	
4.00	<.001	-16	-2	20	R. caudate
3.08	0.001	-18	-78	16	Cuneus (Occipital Ctx) (18)
2.90	0.003	34	28	32	R. middle frontal gyrus
2.73	0.004	-44	-42	0	L. middle temporal gyrus
2.49	0.007	-48	-72	12	Visual association (19)
2.46	0.007	24	-100	-4	Visual association (18)
2.45	0.007	28	-90	12	

Regions in bold indicate area of greatest difference with a contiguous cluster of significant voxels.

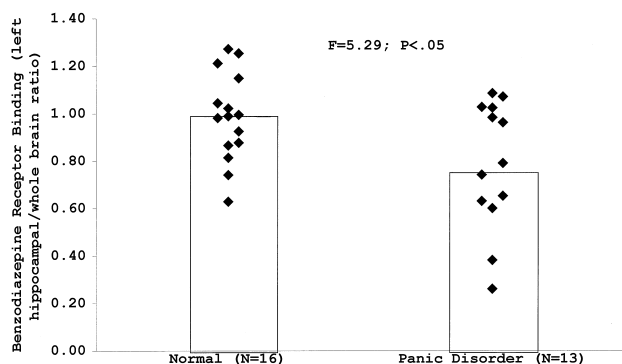


Figure 3. Left hippocampal DV based on measurements obtained from regions of interest placed on the subject's coregistered MRI scans and transferred to the SPECT DV image. There was a 20% reduction in left hippocampal normalized to whole brain benzodiazepine receptor binding (DV) in panic disorder patients (.79 [27 SD] vs. controls .99 [19 SD]) ($F = 5.29$; $df = 1,28$; $p < .05$).

phenomenon. There also were lower global decreases in benzodiazepine receptor binding in patients who had a panic attack at the time of the scan. A significant correlation was also found between panic symptomatology and measures of benzodiazepine receptor binding (DV) in lingual gyrus, in the region of the posterior parahippocampal region (Table 3). This area was not hypothesized to be related to panic symptomatology. An MRI-based region of interest comparison of DV between panicking and non-panicking patients showed lower DV at the time of the scan in the panicking group in left hippocampus (8.4 [5.8 SD] vs. 33.8 [22.9 SD]) ($t = 2.7$; $df = 11$; $p < .05$), left precuneus (14.7 [8.2 SD] vs. 57.2 [36.4 SD]) ($t = 2.8$; $df = 11$; $p < .05$), and right precuneus (13.8 [7.5 SD] vs. 60.6 [37.6 SD]) ($t = 3.0$; $df = 11$; $p < .05$). There was not a significant difference for lingual gyrus (16.3 [9.2 SD] vs. 49.3 [35.6 SD]) ($t = 2.2$; $df = 11$; $p = .07$). There were no significant correlations with general anxiety level as measured by the Hamilton Anxiety Scale or the CRAS.

There was no difference in benzodiazepine receptor binding in left hippocampus (DV normalized to whole brain binding) in women ($n = 18$) compared to men ($n = 11$) in this study (.90 [25 SD] vs. .88 [25 SD]). Similarly there were no gender differences for whole brain binding or other regions (lingual gyrus, prefrontal cortex, precuneus) measured in this study.

Discussion

Panic disorder patients had a decrease in measures of benzodiazepine receptor binding (Distribution Volume [DV]) in left hippocampus measured with SPECT [123 I]iomazenil relative to comparison subjects. Patients who had a panic attack at the time of the scan had a

decrease in benzodiazepine receptor binding in prefrontal cortex (Brodmann's areas 8, 9, and 10) compared to panic patients who did not, suggesting a role for this area in state panic symptom level. These findings were confirmed by both spm and MRI-based region of interest analyses. Exploratory analyses showed a decrease in precuneus in panic compared to controls.

There are several possible explanations for alterations in benzodiazepine receptor binding in panic disorder as measured in the current study. The neuroreceptor imaging techniques employed here derive a measure of the ratio of benzodiazepine receptor binding and affinity, therefore a decrease in either binding or affinity could result in decreases in the measures of benzodiazepine receptor binding reported here. Changes in receptor binding could be related to long-term changes in receptor binding (related to receptor downregulation with the chronic disorder) or to genetic constitution, alternatively state changes in anxiety level at the time of the scan could influence receptor binding. Changes in endogenous benzodiazepine-like compounds (the existence of which remain controversial) or in GABA transmission could also affect the benzodiazepine receptor binding measures in this study. It is also possible that a loss of neurons in specific brain regions could result in a reduction in binding. For hippocampus, however, we have not found a reduction in volume based on a volumetric study comparing panic disorder patients to controls (M. Narayan et al, unpublished data, 1999). The results are not explainable as differences in blood flow as the equilibrium method eliminates differences in flow and tracer delivery from effecting the outcome measure of receptor binding.

The findings of the current study are consistent with preclinical studies showing a decrease in benzodiazepine receptor binding in hippocampus with acute and chronic stress, but not in other regions including pons, striatum, thalamus, cerebellum, or midbrain. The hippocampus, that plays an important role in declarative memory, has long been felt to be involved in the emotions of fear and anxiety (Gray 1982). Lesion studies are consistent with a role for the hippocampus in emotional responses to the context of a fear-inducing situation (Kim and Fanselow 1992; Phillips and LeDoux 1992). Animal strains with genetically low levels of benzodiazepine receptor binding in the hippocampus have an increase in fearful behaviors (Robertson et al 1978). The hippocampus has been implicated in the stress-related disorders (Bremner et al 1995a, 1995b, 1997b; McEwen et al 1992; Sapolsky et al 1990), and stress has been hypothesized to play an etiologic role in the genesis of panic and anxiety. PET studies of blood flow and metabolism in patients with panic disorder found alterations in function of brain areas adjacent to the hippocampus (e.g., parahippocampal gyrus) with findings

Table 3. Areas of Negative Correlation between Benzodiazepine Receptor Binding (DV) and Anxiety Scores (Panic Attack Symptom Scale) in Panic Disorder Patients ($n = 13$)

Z score	<i>p</i> -value	Talairach coordinates			Brain region
		x	y	z	
3.10	0.001	2	56	24	Superior frontal gyrus
2.79	0.003	18	42	36	
3.03	0.001	2	36	44	Medial frontal gyrus (8)
2.86	0.002	10	40	40	
2.60	0.005	-8	46	36	
2.99	0.001	-2	-82	-4	Lingual gyrus (parahippocampus)
2.95	0.002	8	-84	-4	
2.61	0.005	-18	-104	-8	Primary visual cortex (17)
2.53	0.006	-42	-38	4	Middle Temporal Gyrus

Regions in bold indicate area of greatest difference with a contiguous cluster of significant voxels.

of greater decrease in left versus right hippocampus (Nordahl et al 1990; Reiman et al 1984, 1986). Those prior findings are consistent with the results of the current study.

A decrease in measures of benzodiazepine receptor binding (DV) in the area of the precuneus in panic disorder patients compared to controls was found on spm analysis but not confirmed on region of interest analysis. There were significantly lower measures of benzodiazepine receptor binding in the panicing versus nonpanicing patients in this region as assessed with the region of interest analysis. This area is immediately adjacent to posterior cingulate, that has been implicated in anxiety and stress symptoms in previous PET studies (Bench et al 1993; Bremner et al 1998). Precuneus is similar to the hippocampus in having been implicated in memory function (Fletcher et al 1996; Shallice et al 1994). Memory plays a critical role in survival. For example, rapid recall of a potential predator may be life-saving in an acutely dangerous situation. The concept that anxiety represents an abnormality in the brain's fear response system (Charney et al 1996) predicts that alterations in brain areas responsible for memory (including hippocampus and precuneus) will play a role in the pathogenesis of anxiety disorders (Bremner et al 1995a). Findings of decreased benzodiazepine receptor binding in panic disorder in this region were not hypothesized a priori and were based on exploratory analyses. These findings require replication.

The current study found a relationship between elevated levels of anxiety and decreased values for our measure of benzodiazepine receptor binding (DV) in frontal cortex (areas 8, 9, and 10), another brain area involved in memory that was implicated in animal studies of stress and benzodiazepine receptor binding. These frontal cortical areas play an important role in working memory, verbal

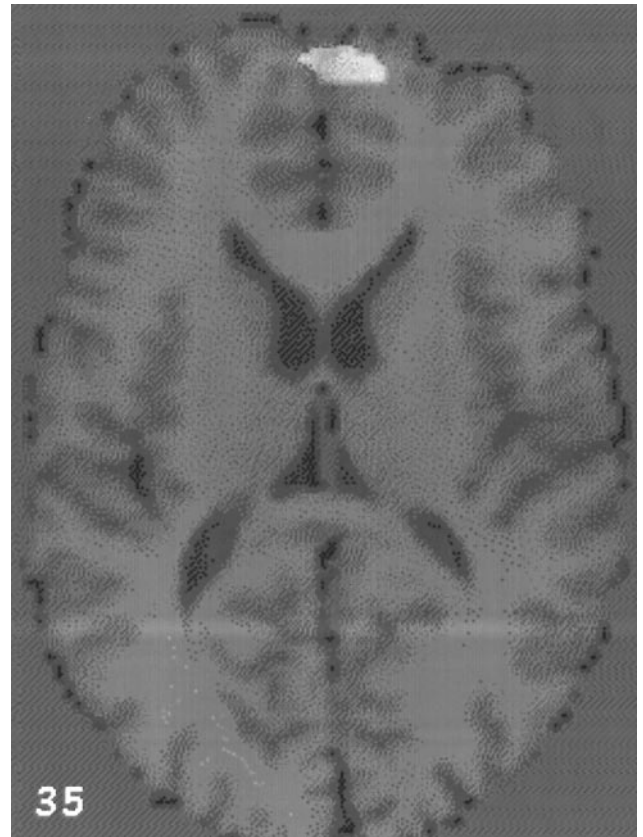


Figure 4. Statistical parametric map overlaid upon a sagittal MR template image showing areas of negative correlation between benzodiazepine receptor binding (DV) and anxiety symptomatology (measured with the Panic Attack Symptom Scale) in prefrontal cortex in patients with panic disorder ($n = 13$). There were significant correlations in the superior and middle frontal gyri (Brodmann's areas 8, 9, and 10), lingual gyrus and primary visual cortex. Only frontal cortex was hypothesized to be related to anxiety levels a priori.

declarative memory, executive control and selection for action (Goldman-Rakic 1988). A prior SPECT blood flow study found a relationship between decreased frontal cortical function and increased anxiety in patients with panic disorder (Woods et al 1988). The current study also found a relationship between decreased benzodiazepine receptor binding and increased anxiety symptom level in lingual gyrus and middle temporal gyrus as well as primary visual cortex. Lingual gyrus is involved in visual memory and memory for faces (Kapur et al 1994). Middle temporal gyrus has been implicated in several studies of declarative memory (Tulving et al 1994) whereas visual cortex mediates perception of visual information.

Other studies have found decreased benzodiazepine receptor binding in panic disorder in orbitofrontal cortex and insula as well as in global measures of binding (Malizia et al 1998). The area of prefrontal cortex (Brodmann's areas 8, 9, and 10) identified in the current study

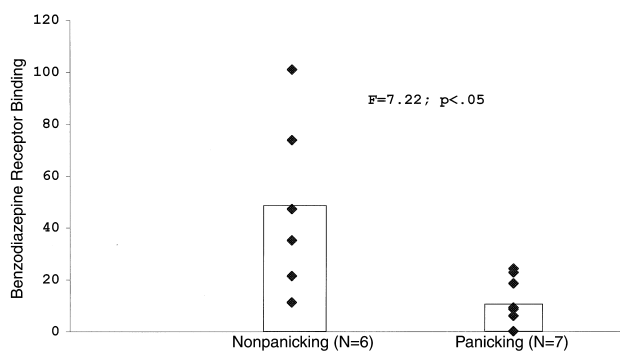


Figure 5. Benzodiazepine receptor binding (DV) in prefrontal cortex (BA8,9) in panic disorder patients with ($n = 7$) and without ($n = 6$) a panic attack at the time of the scan (measured with the Panic Attack Symptom Scale and based on DSM-IV criteria). Benzodiazepine receptor binding (DV) was lower in patients with a panic attack (12.9 [9.1 SD]) compared to patients without a panic attack (48.3 [33.8 SD]) at the time of the scan ($F = 7.22$; $df = 1,12$; $p < .05$). Benzodiazepine receptor binding was 274% lower in the panicking patients compared to nonpanicking patients in this area. The fact that DV in the panic disorder patients as a whole (29.3 [29.2 SD]) was no different from the healthy controls (27.3 [21.6 SD]) suggests that this is a state phenomenon related to anxiety level at the time of the scan.

is more superior to orbitofrontal cortex and insula; however, a number of neuroimaging studies have implicated medial portions of prefrontal cortex extending from orbitofrontal (most inferior) superior to BA 9 in the mediation of mood and anxiety disorders as well as normal emotion. Although we did not find differences in global benzodiazepine receptor binding we did find that patients experiencing a panic attack at the time of the scan had lower levels of benzodiazepine receptor binding than panic disorder patients who did not have a panic attack at the time of the scan. These results suggest that decreases in global benzodiazepine receptor binding play a role in the panic attack symptom states.

Several techniques have evolved for quantitation of neuroreceptor binding with PET and SPECT in human subjects. Most studies have used PET imaging with kinetic techniques that involve bolus injection of radioisotope and kinetic modeling of transfer between brain and blood compartments for measurement of receptor binding parameters. PET in comparison to SPECT has several advantages including higher sensitivity, better resolution, improved methods of attenuation correction. Advantages of SPECT include lower cost, longer half life of radioisotopes (i.e., 13 hours for ^{123}I vs. 20 min for ^{11}C) that improves counting statistics and ease of radiosynthesis, and wider availability. We have performed direct comparisons of quantitation with PET and SPECT using kinetic methods and found good agreement within the same healthy subjects (Bremner et al, in

press). We have also found good agreement between values of benzodiazepine receptor distribution volumes measured with SPECT kinetic and equilibrium techniques (Abi-Dargham et al 1994).

In the current study we used SPECT equilibrium techniques for quantitation of neuroreceptor binding. In this technique, distribution volume is calculated as the ratio of activity in the brain over activity in plasma when transfer on and off the receptor is at equilibrium. It is assumed that equilibrium of binding on and off of receptor is obtained when there is no change in brain activity in serial scans (as was done in the current study). The method assumes that there is perfect equilibration between free activity in plasma and non-receptor-bound activity in the brain. Based on this assumption, free activity in plasma is used as a measure of non-receptor-bound activity in the brain. The method also assumes the nonspecific binding in the brain is negligible (given the 20:1 ratio of specific to nonspecific binding of this radioligand). Therefore, all activity in the brain is considered to represent specifically bound activity. The measure of distribution volume (DV) (or specifically bound activity) is derived from the ratio of “bound” activity in brain at equilibrium divided by “free” activity in plasma. Changes in DV may be related to changes in neuroreceptor number or affinity; however, other factors, such as changes in endogenous ligands competing for the receptor site, could influence this parameter. Violations of any of these assumptions may influence quantitation of neuroreceptor binding. This should, however, not have an effect on specific brain regions more than others, or affect panic disorder patients more than controls.

There are some limitations of the current study that are worth mentioning. Although an attempt was made to match patients and controls for a variety of factors, there was a slightly greater proportion of females in the panic disorder group than in the healthy subject group (although this was not statistically significant). We did, however, not find a gender-based difference in benzodiazepine receptor binding in any of the regions measured in this study. Second, the population of panic disorder patients was recruited by advertisement and therefore may not be generalizable to other panic disorder populations. Most clinical samples of panic disorder, however, are treated with medications that have effects on benzodiazepine receptor binding. Some of the regions reported in this study, such as the hippocampus, are small relative to the anatomical resolution of the SPECT image. All of the findings in this study should be considered exploratory and in need of replication due to the limitations of generalization from animal studies of chronic stress to a clinical psychiatric disorder such as panic disorder.

This study uses statistical parametric mapping (SPM) in a relatively novel way for analysis of neuroreceptor data. Statistical parametric mapping has been widely utilized in the analysis of functional brain imaging studies, and only recently has been applied to neuroreceptor data (Friston et al 1997; Koepp et al 1996; Malizia et al 1998). Statistical parametric mapping and ROI analysis methods offer several relative advantages and disadvantages. Region of interest analyses can be standardized and if coupled with MR have some degree of anatomical certainty. Statistical parametric mapping, on the other hand, allows a broad survey of the brain not available with traditional ROI techniques. This may permit the detection of changes in areas that might not otherwise be assessed. Even in areas which are surveyed by ROIs, areas of difference may involve only subportions of the region which are “diluted” by traditional ROIs (but not by SPM). Statistical parametric mapping, however, has inherent limitations related to problems with multiple comparisons related to the statistical comparison of large numbers of voxels (or individual picture elements) between groups. This has traditionally been addressed by restricting analyses to hypothesized regions, as was done in the current study. In the current study we used MR-based regions of interest on coregistered scans to confirm the findings of the spm analyses.

Although we predicted no areas to have increased binding, the right caudate and cuneus actually demonstrated elevated binding levels. The pathophysiological significance of these areas of increased binding is yet to be determined. These findings need to be replicated in future studies.

The authors would like to thank Helen Sayward, MS, Eric Anderson, and Jennifer Valenti, BS, for image processing and data analysis, Sandi Capelli, RN, Gail Banks, RN, and Louise Brenner, RN, for patient recruitment and assessment, Michelle Early, CNMT, Gary Wyzniewski, CNMT, and Eileen Smith, CNMT, for assistance in SPECT acquisition, Hedy Sarofin for assistance in MRI acquisition, and Louis Amici, MS, and Melyssa Madrak for assistance in radiosynthesis and plasma analysis.

References

- Abi-Dargham A, Laruelle M, Seibyl J, Rattner Z, Baldwin RM, Zoghbi SS, et al (1994): SPECT measurement of benzodiazepine receptors in human brain with [¹²³I]iomazenil: Kinetic and equilibrium paradigms. *J Nucl Med* 35:228–238.
- Abi-Dargham A, Gandelman M, Zoghbi SS, Laruelle M, Baldwin RM, Randall P, et al (1995): Reproducibility of SPECT measurement of benzodiazepine receptors in human brain with [¹²³I]iomazenil. *J Nucl Med* 36:167–175.
- Bench CJ, Friston KJ, Brown RG, Frackowiak RS, Dolan RJ (1993): Regional cerebral blood flow in depression measured by positron emission tomography: The relationship with clinical dimensions. *Psychol Med* 23:579–590.
- Braestrup C, Nielsen M, Nielsen EB, Lyon M (1979): Benzodiazepine receptors in the brain as affected by different experimental stresses: The changes are small and not unidirectional. *Psychopharmacology* 65:273–277.
- Bremner JD, Baldwin R, Horti A, Staib LH, Ng CK, Tan P-Z, et al (in press): Quantitation of benzodiazepine receptor binding with PET [¹¹C]iomazenil and SPECT [¹²³I]iomazenil: Preliminary results of a direct comparison in healthy human subjects. *Psychiatry Res*.
- Bremner JD, Bronen RA, de Erasquin G, Vermetten E, Staib L, Ng CK, et al (1998): Development and reliability of a method for using magnetic resonance imaging for the definition of regions of interest for positron emission tomography. *Clin Posit Imaging* 1:145–159.
- Bremner JD, Horti A, Staib LH, Zea-Ponce Y, Soufer R, Charney DS, et al (1999): Kinetic modeling of benzodiazepine receptor binding with PET and high specific activity [¹¹C]iomazenil in healthy human subjects. *Synapse* (in press).
- Bremner JD, Innis RB, Salomon RM, Staib L, Ng CK, Miller HL, et al (1997a): PET measurement of cerebral metabolic correlates of depressive relapse. *Arch Gen Psychiatry* 54:364–374.
- Bremner JD, Krystal JH, Southwick SM, Charney DS (1995a): Functional neuroanatomical correlates of the effects of stress on memory. *J Trauma Stress* 8:527–554.
- Bremner JD, Randall P, Scott TM, Bronen RA, Seibyl JP, Southwick SM, et al (1995b): MRI-based measurement of hippocampal volume in combat-related posttraumatic stress disorder. *Am J Psychiatry* 152:973–981.
- Bremner JD, Randall P, Vermetten E, Staib L, Bronen RA, Mazure CM, et al (1997b): MRI-based measurement of hippocampal volume in posttraumatic stress disorder related to childhood physical and sexual abuse: A preliminary report. *Biol Psychiatry* 41:23–32.
- Charney DS, Nagy LM, Bremner JD, Goddard AW, Yehuda R, Southwick SM (1996): Neurobiological mechanisms of human anxiety. In: Fogel BS, Schiffler RB, Rao SM, editors. *Neuropsychiatry*, Baltimore: Williams & Wilkins, pp 257–278.
- Dorow R, Horowski R, Paschelke G, Amin M, Braestrup C (1983): Severe anxiety induced by FG7142, a β -carboline ligand for benzodiazepine receptors. *Lancet* 2(8341):98–99.
- Drugan RC, Basile AC, Crawley JN, Paul SM, Skolnick P (1986): Inescapable shock reduces [³H]Ro 5-4864 binding to peripheral type benzodiazepine receptors in the rat. *Pharm Biochem Behav* 24:1673–1677.
- Drugan RC, Morrow AL, Weizman R, Weizman A, Deutsch SI, Crawley JN, et al (1989): Stress-induced behavioral depression in the rat is associated with a decrease in GABA receptor-mediated chloride ion flux and brain benzodiazepine receptor occupancy. *Brain Res* 487:45–51.
- Fletcher PC, Shallice T, Frith CD, Frackowiak RS, Dolan RJ (1996): Brain activation during memory retrieval: The influence of imagery and semantic cueing. *Brain* 119:1587–1596.
- Friston K, Frith C, Liddle P, Frackowiak R (1991): Comparing functional (PET) images: The assessment of significant change. *J Cereb Blood Flow Metab* 11:690–699.
- Friston KJ, Malizia AL, Wilson S, Cunningham VJ, Jones T, Nutt DJ (1997): Analysis of dynamic radioligand displacement or “activation” studies. *J Cereb Blood Flow Metab* 17:80–93.

- Goldman-Rakic PS (1988): Topography of cognition: Parallel distributed networks in primate association cortex. *Ann Rev Neurosci* 11:137–156.
- Gray JA (1982): *The Neuropsychology of Anxiety*. New York: Oxford University Press.
- Guidotti A, Baraldi M, Leon A, Costa E (1990): Benzodiazepines: A tool to explore the biochemical and neuro-physiological basis of anxiety. *FASEB J* 39:1039–1042.
- Hamilton M (1959): The assessment of anxiety states by rating. *Br J Med Psychol* 32:50.
- Havoundjian H, Paul SM, Skolnick P (1986): Rapid, stress-induced modification of the benzodiazepine receptor-coupled chloride ionophore. *Brain Res* 375:401–406.
- Innis RB, Al-Tikriti MS, Zoghbi SS, Baldwin RM, Sybirska EH, Laruelle MA, et al (1991): SPECT imaging of the benzodiazepine receptor: Feasibility of in vivo potency measurements from stepwise displacement curves. *J Nucl Med* 32:1654–1761.
- Jiang H, Robb RA, Holton KS (1992): A new approach to 3-D registration of multimodality medical images by surface matching. In: *Proceedings of the Second Conference on Visual Biomedical Computing*, 196–213.
- Kapur S, Craik FIM, Tulving E, Wilson AA, Houle S, Brown GM (1994): Neuroanatomical correlates of encoding in episodic memory: Levels of processing effect. *PNAS* 91:2008–2011.
- Kascka W, Feistel H, Ebert D (1995): Reduced benzodiazepine receptor binding in panic disorders measured by iomazenil SPECT. *Psychiatry Res* 29:427–434.
- Kim JJ, Fanselow MS (1992): Modality-specific retrograde amnesia of fear. *Science* 256:675–677.
- Koepp MJ, Richardson MP, Brooks DJ, Poline JB, Van Paesschen W, Friston KJ, et al (1996): Cerebral benzodiazepine receptors in hippocampal sclerosis: An objective in vivo analysis. *Brain* 119:1677–1687.
- Kuikka JT, Pitkanen A, Lepola U, Partanen K, Vainio P, Bergstrom KA, et al (1995): Abnormal regional benzodiazepine receptor uptake in the prefrontal cortex in patients with panic disorder. *Nucl Med Comm* 16:273–280.
- LeFur G, Guilloux F, Mitrani N, Mizoule J, Uzan A (1979): Relationship between plasma corticosteroids and benzodiazepines in stress. *J Pharm Exp Ther* 211:305–308.
- Lippa AS, Klepner CA, Yungler L, Sano MC, Smith WV, Beer B (1987): Relationship between benzodiazepine receptors and experimental anxiety in rats. *Pharm Biochem Behav* 9:853–856.
- McEwen BS, Angulo J, Cameron H, Chao HM, Daniels D, Gannon MN, et al (1992): Paradoxical effects of adrenal steroids on the brain: Protection versus degeneration. *Biol Psychiatry* 31:177–199.
- Malizia AL, Cunningham VJ, Bell CJ, Liddle PF, Jones T, Nutt DJ (1998): Decreased brain GABA_A benzodiazepine receptor binding in panic disorder. *Arch Gen Psychiatry* 55:715–720.
- Medina JH, Novas ML, De Robertis (1983a): Changes in benzodiazepine receptors by acute stress: Different effect of chronic diazepam or Ro15–1788 treatment. *Eur J Pharmacol* 96:181–185.
- Medina JH, Novas ML, Wolfman CNV, De Stein ML, De Robertis E (1983b): Benzodiazepine receptors in rat cerebral cortex and hippocampus undergo rapid and reversible changes after acute stress. *Neuroscience* 9:331–335.
- Miller LG, Thompson ML, Greenblatt DJ, Deutsch SI, Shader RI, Paul SM (1987): Rapid increase in brain benzodiazepine receptor binding following defeat stress in mice. *Brain Res* 414:395–400.
- Nordahl TE, Semple WE, Gross M, Mellman TA, Stein MB, Goyer P, et al (1990): Cerebral glucose metabolic differences in patients with panic disorder. *Neuropsychopharmacology* 3:261–271.
- Nutt DJ, Glue P, Lawson C, Wilson S (1990): Flumazenil provocation of panic attacks: Evidence for altered benzodiazepine receptor sensitivity in panic disorder. *Arch Gen Psychiatry* 47:917–925.
- Phillips RG, LeDoux JE (1992): Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behav Neurosci* 106:274–285.
- Reiman EM, Lane RD, Ahern GL, Schwartz GE, Davidson RJ, Friston KJ, et al (1997): Neuroanatomical correlates of externally and internally generated human emotion. *Am J Psychiatry* 154:918–925.
- Reiman E, Raichle ME, Robins, Mintun MA, Fusselman MJ, Fox PT, et al (1986): The application of positron emission tomography to the study of panic disorder. *Am J Psychiatry* 143:469–477.
- Reiman EM, Raichle ME, Butler FK, Herscovitch P, Robins E (1984): A focal brain abnormality in panic disorder: A severe form of anxiety. *Nature* 310:683–685.
- Robertson HA, Martin IL, Candy JM (1978): Differences in benzodiazepine receptor binding in Maudsley-reactive and nonreactive rats. *Eur J Pharmacol* 50:455–457.
- Rocca P, Ferrero P, Gualerzi A, Zanalda E, Maina G, Bergamasco B, et al (1992): Peripheral type benzodiazepine receptors in anxiety disorders. *Acta Psychiatr Scand* 84:537–544.
- Roy-Byrne PP, Cowley DS, Greenblatt DJ, Shader RI, Hommer D (1990): Reduced benzodiazepine sensitivity in panic disorder. *Arch Gen Psychiatry* 47:534–538.
- Roy-Byrne PP, Lewis N, Villacres E, Diem H, Greenblatt DJ, Shader RI, et al (1989): Preliminary evidence of benzodiazepine subsensitivity in panic disorder. *Biol Psychiatry* 26:744–748.
- Sapolsky RM, Uno H, Rebert CS, Finch CE (1990): Hippocampal damage associated with prolonged glucocorticoid exposure in primates. *J Neurosci* 10:2897–2902.
- Schlegel S, Teinert H, Bockisch A, Hahn K, Schloesser R, Benkert O (1994): Decreased benzodiazepine receptor binding in panic disorder measured by iomazenil-SPECT. A preliminary report. *Eur Arch Psych Clin Neurosci* 244:49–51.
- Shallice T, Fletcher PC, Frith CD, Grasy P, Frackowiak RS, Dolan RJ (1994): Brain regions associated with acquisition and retention of verbal episodic memory. *Nature* 368:633–635.
- Skerritt JH, Trisdikoon P, Johnston GAR (1981): Increased GABA binding in mouse brain following acute swim stress. *Brain Res* 215:398–403.
- Spitzer RL, Williams JBW, Gibbon M (1987): Structured clinical interview for DSM-III-R. New York State Psychiatric Institute, Biometrics Research Department, New York.
- Sybirska E, Seibyl JP, Bremner JD, Baldwin RM, Al-Tikriti MS, Bradberry C, et al (1993): [¹²³I]Iomazenil SPECT imaging

- demonstrates significant benzodiazepine receptor reserve in human and non-human primate brain. *Neuropharmacology* 32:671–680.
- Talairach J, Tournoux P (1988): *Co-Planar Stereotaxic Atlas of the Human Brain: 3-Dimensional Proportional System: An Approach to Cerebral Imaging*. Stuttgart-New York: Thieme-Verlag.
- Tulving E, Kapur S, Craik FIM, Moscovitch M, Houle S (1994): Hemispheric encoding/retrieval asymmetry in episodic memory: Positron emission tomography findings. *PNAS* 91:2016–2020.
- Weizman A, Weizman R, Kook KA, Vocci F, Deutsch SI, Paul SM (1990): Adrenalectomy prevents the stress-induced decrease in in vivo [³H]Ro 15-1788 binding to GABA_A benzodiazepine receptors in the mouse. *Brain Res* 519:347–350.
- Weizman R, Laor N, Karp L, Dagan E, Reiss A, Dar DE, et al (1994): Alteration of platelet benzodiazepine receptors by stress of war. *Am J Psychiatry* 151:766–767.
- Weizman R, Tanne Z, Granek M, Karp L, Golomb M, Tyano S, et al (1987): Peripheral benzodiazepine binding sites on platelet membranes are increased during diazepam treatment of anxious patients. *Eur J Pharmacol* 138:289–292.
- Weizman R, Weizman A, Kook KA, Vocci F, Deutsch SI, Paul SM (1989): Repeated swim stress alters brain benzodiazepine receptors measured in vivo. *J Pharm Exp Ther* 249:701–707.
- Woods SW, Charney DS, Silver JM, Krystal JH, Heninger GR (1991): Behavioral, biochemical and cardiovascular responses to the benzodiazepine receptor antagonist flumazenil in panic disorder. *Psychiatry Res* 36:115–124.
- Woods SW, Koster K, Krystal JH, Smith EO, Zubal IG, Hoffer PB, et al (1988): Yohimbine alters regional cerebral blood flow in panic disorder (letter). *Lancet* 2:678.
- Zoghbi SS, Baldwin RM, Seibyl JP, Al-Tikriti MS, Zea-Ponce Y, Laruelle M, et al (1992): Pharmacokinetics of the SPECT benzodiazepine radioligand [¹²³I]iomazenil in human and nonhuman primates. *Nucl Med Biol* 19:881–888.