

# No Evidence of Altered *In Vivo* Benzodiazepine Receptor Binding in Schizophrenia

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Deficits in gamma-amino-butyric acid (GABA) neurotransmitter systems have been implicated in the pathophysiology of schizophrenia for more than two decades. Previous postmortem and *in vivo* studies of benzodiazepine (BDZ) receptor density have reported alterations in several brain regions of schizophrenic patients. The goal of this study was to better characterize possible alterations of the *in vivo* regional distribution volume ( $V_T$ ) of BDZ receptors in schizophrenia, using the selective BDZ antagonist [ $^{123}$ I]iomazenil and single photon emission computerized tomography (SPECT). Regional BDZ  $V_T$  was measured under sustained radiotracer equilibrium conditions. The reproducibility and reliability

of this measurement was established in four healthy volunteers. No differences in regional BDZ  $V_T$  were observed between 16 male schizophrenic patients and 16 matched controls. No relationships were observed between BDZ  $V_T$  and severity of psychotic symptoms in any of the regions examined. In conclusion, this study failed to identify alterations of BDZ receptors density in schizophrenia. If this illness is associated with deficits in GABA transmission, these deficits do not substantially involve BDZ receptor expression or regulation. [*Neuropsychopharmacology* 20:650-661, 1999] © 1999 American College of Neuropsychopharmacology. Published by Elsevier Science Inc.

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Alterations of gamma-amino-butyric acid (GABA) system have been implicated in the pathophysiology of schizophrenia for more than two decades. The recognition that GABA has an inhibitory effect on dopamine transmission prompted several authors to postulate

that disinhibition of dopamine function in schizophrenia may stem from a deficit in inhibitory, GABA mediated systems (Roberts 1972; Stevens 1975; van Kammen 1977). In addition, postmortem studies have reported alterations in regional concentrations of several markers of the GABAergic system in schizophrenia.

In accordance with the hypothesis of a GABA deficit in schizophrenia, GABAergic cells and markers of GABAergic cells have usually been reported to be decreased in postmortem studies in patients with schizophrenia (Bird et al. 1977; Perry et al. 1979; Simpson et al. 1989; Reynolds et al. 1990; Benes et al. 1991; Sherman et al. 1991; Akbarian et al. 1995b; Beasley and Reynolds 1997). However, few of these observations have been replicated (Bennett et al. 1979; Cross et al. 1979; Akbarian et al. 1995b; Woo et al. 1997).

As far as postsynaptic markers are concerned, increased binding of the GABA<sub>A</sub> receptor agonist [ $^3$ H]mus-

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cimol has been consistently reported in prefrontal cortex, caudate nucleus, hippocampus and cingulate cortex in schizophrenia (Hanada et al. 1987; Benes et al. 1992; Benes et al. 1996). However, GABA<sub>A</sub> receptor subunits mRNA was unchanged in prefrontal cortex (Akbarian et al. 1995a). Benzodiazepine receptors have been reported to be unchanged in the hippocampus and prefrontal cortex (Reynolds and Stroud 1993; Benes et al. 1997; Pandey et al. 1997), decreased in hippocampus, anterior cingulate cortex, somatomotor cortex, cerebellum, globus pallidus (Squires et al. 1993), and increased in frontal, temporal and putamen (Kiuchi et al. 1989). The increase in [<sup>3</sup>H]muscimol binding to the GABA binding site of the GABA<sub>A</sub>/BDZ receptor complex, in the face of unchanged BDZ receptor binding in some regions of schizophrenic brains, has been interpreted as indicating an uncoupling of GABA<sub>A</sub> and BDZ receptors in schizophrenia (Benes et al. 1997). Thus, the overall picture is suggestive of a functional and/or structural decrease in GABAergic activity, possibly associated with a compensatory regulation of postsynaptic receptors. Still, many contradictory reports have been published, and most of these findings await independent replication.

In contrast to the abundant postmortem literature, very few *in vivo* studies of GABA transmission in schizophrenia have been reported. Busato et al. (1997) using SPECT and the BDZ receptor radiotracer [<sup>123</sup>I]iomazenil, reported no regional differences in the uptake of [<sup>123</sup>I]iomazenil between 15 patients with schizophrenia and 12 healthy controls. In the schizophrenic group, severity of positive symptoms was associated with decreased [<sup>123</sup>I]iomazenil uptake in the left medial temporal region, and severity of negative symptoms was correlated with decreased medial frontal [<sup>123</sup>I]iomazenil uptake. However, these correlations were not significant after Bonferroni correction. Schröder et al. (1997) investigated [<sup>123</sup>I]iomazenil binding in 20 patients with schizophrenia, but no control group was studied. They reported that BPRS scores were correlated with increased [<sup>123</sup>I]iomazenil binding in the medial prefrontal cortex. Both studies were semi-quantitative and, as such, are subject to numerous potential artifacts. Yet, these reports suggested that BDZ receptors density might be associated with symptomatic features of schizophrenia.

In the present study, we used the constant infusion/sustained equilibrium paradigm with SPECT and [<sup>123</sup>I]iomazenil (Laruelle et al. 1993, 1994; Abi-Dargham et al. 1994b; Lassen 1996) to examine possible alterations of BDZ receptor binding potential (BP) in 16 patients with schizophrenia and 16 matched controls, and to study possible relationships of regional BDZ receptor density with symptomatology. In contrast to the semi-quantitative methods used in the above-described studies, the constant infusion/sustained equilibrium method

is insensitive to possible group differences in blood flow and peripheral clearance which affect semi-quantitative methods following single bolus injection (Carson et al. 1992). In addition, regions of interest (ROI) were identified following coregistration with MRI. This method yields several potential outcome measures, depending on the nature of the concentration parameter used in the denominator of the distribution volume ratio. The first goal of this investigation was to assess the reproducibility and reliability of each candidate outcome measure in a test/retest study in four healthy subjects. The second goal was to compare BDZ receptor density between patients with schizophrenia and healthy controls using the most reliable outcome measure.

## MATERIALS AND METHODS

### Subjects

The study was performed according to protocols approved by the Yale School of Medicine and VA Connecticut at West Haven Institutional Review Boards. Patients were recruited from the VA Connecticut at West Haven. Study criteria for patients with schizophrenia were as follows: 1) diagnosis of schizophrenia according to Diagnostic and Statistical Manual (DSM-IV); 2) no other DSM-IV axis I diagnosis; 3) no history of alcohol or substance abuse or dependence; 4) absence of benzodiazepine administration for at least 21 days; 5) no concomitant or past severe medical conditions; 6) no pregnancy; 7) no metallic objects in the body; and 8) ability to provide informed consent. Patient clinical evaluation at intake included the Positive and Negative Symptoms Scales (PANS) (Kay et al. 1987), a scale that includes the Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham 1962), and the AIMS (Munetz and Benjamin 1988). Since BDZ receptor density is not consistently affected by chronic antipsychotic administration (Gavish et al. 1988; Giardino et al. 1991), neuroleptic withdrawal was not required for this study.

Study criteria for healthy controls were: 1) absence of past or present neurological or psychiatric illnesses; 2) absence of concomitant or past severe medical conditions; 3) no pregnancy; 4) no metallic objects in the body; and 5) informed consent. Healthy controls were group matched to patients for age ( $\pm 5$  years), gender, and race. Four healthy controls were scanned twice, at 1 to 2 week intervals, to evaluate the reproducibility and reliability of the measurements.

### Radiolabeling

Sodium [<sup>123</sup>I]iodide (no-carrier-added in 0.1 M NaOH) was purchased from Nordion International, Vancouver, B.C. [<sup>123</sup>I]iomazenil is a high affinity antagonist of the BDZ receptor ( $K_D = 0.35$  nM at 22°C) (Beer et al. 1990;

Laruelle et al. 1994). [<sup>123</sup>I]Iomazenil was prepared by iododestannylation of its tributylstannyl precursor as previously described (Zoghbi et al. 1992; Zea-Ponce et al. 1993) in average yield  $63.3 \pm 10.7\%$  and radiochemical purity  $97.6 \pm 2.0\%$  (with these and subsequent data expressed as mean  $\pm$  S.D.;  $n = 19$ ). Sterility was confirmed by lack of growth in two media: fluid thioglycolate at 35°C and soybean-casein digest at 25°C for two weeks. Apyrogenicity was confirmed by the LAL test (Endosafe, Charleston, NC).

### Data Acquisition

All subjects received potassium iodide (SSKI solution 0.6 g) in the 24 h period prior to scan. Four fiducial markers filled with 10  $\mu$ Ci of [<sup>99m</sup>Tc]NaTcO<sub>4</sub> were attached on both sides of the subject's head at the level of the cantho-meatal line to identify this plane during image analysis. Subjects received a priming bolus of [<sup>123</sup>I]iomazenil ( $3.83 \pm 0.43$  mCi for controls and  $3.85 \pm 0.41$  mCi for patients), followed by a continuous infusion at a constant rate ( $1.01 \pm 0.11$  mCi/h and  $1.02 \pm 0.11$  mCi/h, respectively) using a computer-controlled pump (IMED pump, Jemini PC-1, San Diego, CA). Duration of infusion was 7 h (total injected dose of  $10.68 \pm 1.26$  mCi and  $10.44 \pm 1.16$  mCi for controls and patients, respectively). SPECT scanning was initiated at 6 h into the infusion. Four consecutive 10 min frames were acquired with the Ceraspect camera (Digital Scintigraphics, Waltham, MA), with a resolution in water of 12 mm in the three planes (Zubal et al. 1990). Three venous blood samples were collected in the middle of the scanning session. MRI scans were acquired on the GE 1.5 Tesla Signa super-conducting magnet. A sagittal scout scan was used to define the anterior-posterior commissure (AC-PC). Axial slices in the AC-PC plane were acquired using a double spin echo protocol with TE's of 30 ms and 80 ms, TR 4000 ms, matrix  $256 \times 192$ , 1 NEX, 3 mm thickness, no gap (pixel size  $0.94 \times 0.94$ ). The T2 weighted images (TE 80 ms) were used in this analysis.

### Image Analysis

SPECT images were reconstructed using a Butterworth filter (cutoff = 1 cm, power factor = 10) and displayed on a  $64 \times 64 \times 32$  matrix (pixel size =  $3.33 \times 3.33$  mm, slice thickness = 3.32 mm, voxel volume =  $36.76$  mm<sup>3</sup>) using the Ceraspect software. The four acquisitions were individually inspected for evidence of head movement, and reoriented, if needed, to the first acquisition, using the fiducial markers as landmarks. Occipital counts were measured on each acquisition, and the time activity curve was analyzed to check the quality of the equilibrium (a regional change of less than 10%/h was chosen as equilibrium criteria).

No MRIs were acquired for the test/retest subjects. In

these subjects, the attenuation ellipse and the ROIs were directly drawn on the SPECT images, as previously described for the study of the test/retest reproducibility of BDZ distribution volume measurements by kinetic analysis of single bolus [<sup>123</sup>I]iomazenil injection experiments (Abi-Dargham et al. 1995).

For the clinical study, attenuation correction and ROI identification were performed after coregistration to the MRI. The aligned four SPECT acquisitions were summed on a slice-by-slice basis, and the summed SPECT data set was transferred to the program ANALYZE (Mayo Foundation, Rochester, Minnesota). MRI T2 weighted images were coregistered to the summed SPECT image using a contour-fitting algorithm, after manual tracings of the contour of the brain on both SPECT and MRI images (Jiang and Robb 1992). The coregistered MRI was then used to draw the attenuation correction ellipse around the skull on each slice. Ellipses were transferred to the corresponding SPECT slices, and attenuation correction was performed assuming uniform attenuation equal to that of water, corrected for higher scatter in the center of the object (attenuation coefficient  $\mu = 0.12$  cm<sup>-1</sup>). The attenuation corrected SPECT was then coregistered to the original MRI. Final SPECT voxel volume was  $0.94 \times 0.94 \times 3$  mm = 2.64 mm<sup>3</sup>. This procedure was followed in order to: 1) perform the attenuation correction before any significant rotation or reslicing of the SPECT data; and 2) orient the SPECT slices of all subjects into the AC-PC line for data analysis. ROIs were drawn on each MRI plane by the same rater, blindly to the diagnosis, according to anatomical criteria derived from the Duvernoy atlas (Duvernoy 1991). Sampled ROIs included right and left frontal, cingulate, temporal, parietal, and occipital cortices, right and left hippocampus, amygdala, parahippocampal gyrus, thalamus, striatum, and cerebellum. A region was also drawn on the midbrain white matter, which is the region with the lowest density of BDZ receptors in the brain. Cortical ROIs included both gray and white matter. These ROIs were then transferred to the corresponding SPECT planes. Average cpm/pixel ROI activities were decay corrected and expressed in  $\mu$ Ci/cc using an average calibration factor derived from <sup>123</sup>I distributed source phantom studies performed at regular intervals throughout the duration of the studies. Because reduced [<sup>3</sup>H]nipecotic acid binding to GABA transporters has been reported to be more prominent in the left than the right temporal regions, right and left regions were analyzed separately. A laterality index was calculated as  $(L - R)/(L + R)$ . This index varies from  $-1$  (all to the right) to  $+1$  (all to the left).

### Plasma Analysis

Blood samples were analyzed as previously described (Zoghbi et al. 1992). A 200  $\mu$ L aliquot of the plasma was

counted to measure the plasma activity concentration ( $CP_{ACT}$ ,  $\mu\text{Ci/mL}$ ). Extraction (ethyl acetate) was followed by reverse phase HPLC to measure the metabolite-corrected total plasma activity (parent compound plasma concentration,  $CP_{PAR}$ ,  $\mu\text{Ci/mL}$ ). Plasma protein binding was measured by ultrafiltration through Centrifree membrane filters (Amicon Division, W.R. Grace & Co., Danvers, MA) (Gandelman et al. 1994). One aliquot from a pooled sample of plasma obtained from different healthy volunteers was processed as a standard with each experiment to control for day to day variability in the free fraction assay. The plasma free fraction ( $f_1$ ) measured in the subject sample was corrected for inter-assay variability using the plasma free fraction measured in the standard ( $f_{1\text{ std}}$ ) and the average of the standard measurement over the course of the study ( $f_{1\text{ ave}}$ ,  $0.39 \pm 0.03$ ) according to  $[(f_1 \times f_{1\text{ ave}})/f_{1\text{ std}}]$ . The parent compound plasma concentration ( $CP_{PAR}$ ) was multiplied by  $f_1$  to yield the free plasma [ $^{123}\text{I}$ ]iomazenil concentration ( $CP_{FREE}$ ). Plasma clearance of total activity, total parent [ $^{123}\text{I}$ ]iomazenil and free [ $^{123}\text{I}$ ]iomazenil (L/h) were calculated as the ratio of the rate of infusion ( $\mu\text{Ci/h}$ ) to the corresponding plasma concentrations ( $CP_{ACT}$ ,  $CP_{PAR}$ , and  $CP_{FREE}$ , respectively,  $\mu\text{Ci/L}$ ).

### Outcome Measures

At equilibrium, the ligand bound to the receptors ( $B$ ) is related to receptor parameters (maximal density of available receptors,  $B_{\text{max}}$ , and equilibrium dissociation constant,  $K_D$ ) and to the free tracer concentration (Free) by the Michaelis-Menten equation (Equation 1)

$$B = \frac{B_{\text{max}} \times \text{Free}}{K_D + \text{Free}} \quad (1)$$

When experiments are carried out at tracer doses, Free is negligible compared to  $K_D$ , and Equation 1 can be simplified as follows (Equation 2)

$$B = \frac{B_{\text{max}} \times \text{Free}}{K_D} \quad (2)$$

The binding potential (BP) quantifies receptor availability at tracer doses, and corresponds to the product of receptor density and affinity ( $B_{\text{max}}/K_D$ ). Rearranging Equation 2 shows that BP is equal to the Bound/Free ratio at equilibrium (Equation 3)

$$\text{BP} = \frac{B}{\text{Free}} = \frac{B_{\text{max}}}{K_D} \quad (3)$$

In vivo,  $B$  is usually derived by subtracting the activity in a region of reference ( $C_{\text{REF}}$ ) from the activity in a region of interest ( $C_{\text{ROI}}$ ).  $C_{\text{REF}}$  is assumed to provide an estimate of the free and nonspecific binding in the region of interest, so that  $C_{\text{ROI}} - C_{\text{REF}} = B$ . The measurement of the free requires plasma analysis. When this measurement is not performed,  $C_{\text{REF}}$  can be used as the denominator (i.e., concentration parameter) in the oper-

ational equation used to yield a practical measure of binding potential ( $BP_{\text{OP}}$ ) (Equation 4)

$$\text{BP}_{\text{OP}} = \frac{C_{\text{ROI}} - C_{\text{REF}}}{C_{\text{REF}}} \quad (4)$$

Measurement of activity in a region devoid of receptors ( $C_{\text{REF}}$ ) is required for this method. For [ $^{123}\text{I}$ ]iomazenil, the white matter activity has been used as measure of  $C_{\text{REF}}$  (Busatto et al. 1997). However, close to 50% of white matter "nonspecific" activity is activity scattered from adjacent cortical areas, as demonstrated by the fact that 50% of [ $^{123}\text{I}$ ]iomazenil white matter activity is "displaceable" by flumazenil (Abi-Dargham et al. 1994b). The absence of a measurable  $C_{\text{REF}}$  has two consequences for the calculation of BP: 1)  $C_{\text{ROI}}$  is used instead of  $B$  in the numerator, which is adequate since the nonspecific component is negligible (Abi-Dargham et al. 1994b); and 2) the concentration parameter, i.e., the denominator, must be derived from a plasma measurement.

Since passive diffusion is the mechanism of transport of [ $^{123}\text{I}$ ]iomazenil across the blood-brain barrier, free [ $^{123}\text{I}$ ]iomazenil concentration in plasma and brain water are equal under the steady-state conditions achieved during constant infusion (Kawai et al. 1991; Laruelle et al. 1994). Therefore, free [ $^{123}\text{I}$ ]iomazenil concentration in brain water can be estimated by the free plasma [ $^{123}\text{I}$ ]iomazenil concentration ( $CP_{\text{FREE}}$ ). Since the numerator includes both specific binding and nondisplaceable components (nonspecific binding and free), we use the term total distribution volume ( $V_T$ ) rather than BP to characterize the BDZ receptors availability (Equation 5)

$$V_T = \frac{C_{\text{ROI}}}{CP_{\text{FREE}}} \quad (5)$$

While  $CP_{\text{FREE}}$  is theoretically the most appropriate denominator for the distribution volume ratio (Equation 5), the measurement of  $CP_{\text{FREE}}$  is prone to experimental errors. Other candidate denominators are available, which might prove more robust (Table 1). The simplest outcome measure ( $V_{T\text{ INF}}$ ) involves correcting the measured regional activity by the hourly infused dose (HID) ( $V_{T\text{ INF}} = C_{\text{ROI}}/\text{HID}$ ). To take into account between individual differences in the rate of peripheral clearance of the infused activity, regional activity can be expressed relatively to the total plasma activity at equilibrium ( $V_{T\text{ ACT}} = C_{\text{ROI}}/CP_{\text{ACT}}$ ). This outcome measure requires a simple measurement of plasma activity. To account for between individual differences in the metabolism of iomazenil, regional activity can be expressed relatively to the total (free and bound to plasma proteins) plasma concentration of the parent [ $^{123}\text{I}$ ]iomazenil ( $CP_{\text{PAR}}$ ), which requires determination by HPLC of the parent fraction in plasma extracts. This outcome measure has been denoted  $V_T'$  ( $V_T' = C_{\text{ROI}}/CP_{\text{PAR}}$ ) (Abi-Dargham et al. 1994a). Finally, to correct for possible differences in plasma protein binding of the radiotracer,

the measurement of plasma free fraction ( $f_f$ ) must be obtained ( $V_T = C_{ROI}/CP_{FREE}$ ). The choice between these outcome measures can not be made without knowledge of the reproducibility of these measurements, because of the conflicts between potential biases and experimental errors. The more measurements are incorporated into the outcome measure, the greatest the protection against biases but the greatest the vulnerability to propagation of experimental errors. Test/retest studies allow to determine the reproducibility of each outcome measure, as well as their reliability, i.e. their ability to detect between subject differences. We elected to evaluate the variability and reliability of these outcome measures by scanning four healthy subjects twice. The results of the test/retest studies determined the outcome measure used in the clinical investigation.

### Statistical Analysis

All values are expressed as mean  $\pm$  SD. The variability of one measurement between test and retest was computed as the absolute value of the difference between test and retest, expressed in percentage of the mean value of both measurements. Differences in variability between different outcome measures were investigated by a repeated measure ANOVA with regions as cofactor. The reliability of the measurements was assessed relatively to the between subjects variance by the intraclass correlation coefficient,  $\rho$ , calculated as (Kirk 1982)

$$\frac{MSBS - MSWS}{MSBS + (n - 1)MSWS}$$

where MSBS is the mean sum of square between subjects and MSWS is the mean sum of square within subjects. This coefficient estimates the reliability of the

measurement and can vary between 0 (no reliability) and 1 (maximum reliability, achieved in case of identity between test and retest, i.e., MSWS = 0). Group differences in outcome measures were assessed with a two-tailed unpaired t test, using  $p < .05$  as significance level, following an F test to evaluate the assumption of equal variance. The relationship between continuous variables was assessed with Pearson product correlation coefficient, followed by ANOVA.

In addition to the region of interest analysis described above, we also performed a pixel-by-pixel statistical analysis using statistical parametric mapping (SPM 96) (Friston et al. 1995). The advantage of SPM compared to the ROI method is that SPM does not make any prediction regarding the size or location of an area of significant change. Therefore, this method is able to detect areas of change (statistical excursions) limited to small parts of the regions selected by the ROI analysis, as well as areas of change overlapping two regions. The disadvantage of SPM is the inevitable blurring of the images resulting from the spatial normalization procedure, as well as the intrinsic exploratory nature of its results. SPECT images were transformed into BDZ  $V_T$  maps by dividing the activity concentration in each pixel by the steady-state [ $^{123}$ I]iomazenil plasma concentration. MRI scans were mapped into the Talairach space (Talairach and Tournoux 1988) using a 12 parameter affine transformation which was then applied to the subject's BDZ  $V_T$  map, in a concentration conservative manner. The atlas-fitted  $V_T$  maps were smoothed with a Gaussian kernel to minimize the effect of intersubject anatomical variability. Since the  $V_T$  map consists of absolute numbers which are directly comparable between subjects, we performed the SPM analysis without global normalization. The statistical z map

**Table 1.** Definition of Potential Outcome Measures

Notation	Outcome Measure		Factors Taken Into Account (Yes/No)		
	Equation	Equation	Activity clearance	Iomazenil metabolism	Binding to plasma proteins
$V_{TINF}$	Hourly infused dose	$\frac{C_{ROI}}{HIR}$	No	No	No
$V_{TACT}$	Total plasma activity	$\frac{C_{ROI}}{CP_{ACT}}$	Yes	No	No
$V_{T'}$	Total plasma [ $^{123}$ I]iomazenil activity	$\frac{C_{ROI}}{CP_{PAR}}$	Yes	Yes	No
$V_T$	Free plasma [ $^{123}$ I]iomazenil activity	$\frac{C_{ROI}}{CP_{FREE}}$	Yes	Yes	Yes

Abbreviations:  $C_{ROI}$ , [ $^{123}$ I]iomazenil concentration in ROI at equilibrium ( $\mu$ Ci/g of tissue); HIR, hourly infused dose ( $\mu$ Ci/g body weight);  $CP_{ACT}$ , plasma activity concentration (parent and metabolized [ $^{123}$ I]iomazenil, bound and free) ( $\mu$ Ci/ml of plasma);  $CP_{PAR}$ , plasma concentration of parent compound (bound and free) ( $\mu$ Ci/ml of plasma);  $CP_{FREE}$ , plasma concentration of free [ $^{123}$ I]iomazenil ( $\mu$ Ci/ml of plasma); VT, total distribution volume.

threshold was set at 2.53 ( $p = .005$ ). A more traditional SPM analysis using ANCOVA to normalize global  $V_T$  was also performed.

**RESULTS**

**Test/Retest Study**

The test/retest variability and reliability of the plasma measurements are provided in Table 2. The variability was lower than 10% for all measures. Clearance of the parent compound was the measurement with lowest variability,  $4 \pm 3\%$ , and highest reliability 0.93. Correction for free fraction resulted in a slight degradation of both reproducibility and reliability. However, correcting  $f_1$  for between assay variability improved the reproducibility of the measurement (from  $6 \pm 4\%$  to  $3 \pm 3\%$ ).

For the distribution volumes, four regions were examined (frontal, temporal, cingulate and occipital). As no statistically significant differences were observed in reproducibility indices between these regions, values derived from the four regions were averaged for the purpose of comparing the outcome measures (Table 3). Significant differences in reproducibility were observed between outcome measures (ANOVA,  $p = .004$ ):  $V_{T\ ACT}$  had a significantly better reproducibility ( $10 \pm 3\%$ ), compared to  $V_{T\ INF}$  ( $14 \pm 1\%$ ),  $V_{T'}$  ( $13 \pm 1\%$ ), and  $V_T$  ( $15 \pm 1\%$ ). The difference in variability between  $V_{T'}$  and  $V_T$  was not significant ( $p = .38$ ).  $V_T$  showed the highest reliability ( $0.70 \pm 0.05$ ), although between methods differences in reliability were not significant ( $p = .18$ ). Because  $V_T$  was the theoretically preferred outcome measure (Table 1) and the variable with the greatest power to detect inter-individual differences (i.e., highest reliability, Table 3), this outcome measure was selected for the clinical study, despite the fact that its reproducibility was lower than  $V_{T\ ACT}$ .

**Table 2.** Variability and Reliability of Plasma Measurements

Measure	Variability (%)	Reliability ( $\rho$ )
Clearance total activity	$7 \pm 4$	.52
Fraction parent by HPLC	$6 \pm 2$	.93
Clearance parent	$4 \pm 3$	.93
$f_1$ uncorrected <sup>a</sup>	$6 \pm 4$	.54
$f_1$ corrected <sup>b</sup>	$3 \pm 3$	.57
Clearance free (corrected)	$8 \pm 6$	.78

Reproducibility parameters derived from repeated experiments in four healthy subjects.

<sup>a</sup> $f_1$  uncorrected: plasma free fraction measured with ultracentrifugation of the subject's sample.

<sup>b</sup> $f_1$  corrected: plasma free fraction measured with ultracentrifugation of the subject's sample, corrected for between assay variability using internal standards.

**Table 3.** Variability and Reliability of Distribution Volumes

Measure	Variability (%)	Reliability ( $\rho$ )
$V_{T\ INF}$	$14 \pm 1$	.52 $\pm$ .16
$V_{T\ ACT}$	$10 \pm 3^a$	.53 $\pm$ .20
$V_{T'}$	$13 \pm 1$	.66 $\pm$ .06
$V_T$	$15 \pm 1$	.70 $\pm$ .05

Abbreviations:  $V_{T\ INF}$  = distribution volume relative to hourly infused dose;  $V_{T\ ACT}$  = distribution volume relative to total plasma activity at equilibrium.

<sup>a</sup>Significantly different from three other outcome measures, ANOVA ( $p = .004$ ), followed by Fisher PLSD.

**Clinical Study**

The study included sixteen patients and sixteen controls. Both groups were matched on gender (all males), age and ethnicity (Table 4). Eleven patients were taking neuroleptic drugs at the time of the scan, and five patients were neuroleptic-free: three of them had been previously treated and were off neuroleptics for more than 21 days, and two patients were drug naive. The average duration of prior neuroleptic exposure was  $13 \pm 10$  years. Four patients had noticeable tardive dyskinesia (AIMS scores  $> 6$ ).

Table 5 summarizes plasma measurements in both groups. No differences were observed between groups in total activity clearance. However, [<sup>123</sup>I]iomazenil metabolism was slower in controls than in patients (percent parent:  $14 \pm 7\%$  versus  $12 \pm 3\%$  in controls and patients, respectively,  $p = .077$ ). As a result, clearance of the parent compound was slower in controls than in patients ( $116 \pm 23$  L/h versus  $142 \pm 36$  L/h in controls and patients, respectively,  $p = .023$ ). The free fraction was similar in both groups, indicating similar binding to plasma proteins. The difference in rate of metabolism resulted in a slower clearance of the free compound in controls compared to patients ( $370 \pm 86$  L/h versus  $441 \pm 115$  L/h,  $p = .058$ ). In other terms, for a similar rate of infusion, the plasma concentration of [<sup>123</sup>I]iomazenil tended to be lower in the schizophrenic patients than in the controls.

Equilibrium criteria were satisfied for every subject. The distribution of BDZ  $V_T$  was in accordance with the known distribution of BDZ receptors in the human brain (Braestrup et al. 1977; Luabeya et al. 1984). High-

**Table 4.** Sample Demographics

Characteristics	Controls	Schizophrenics
n	16	16
Age (years)	$45 \pm 10$	$44 \pm 10$
Gender	All males	All males
Ethnicity (AA/C/H) <sup>a</sup>	5/10/1	7/8/1
Weight (kg)	$84 \pm 13$	$82 \pm 12$

<sup>a</sup> AA, Afro-Americans; C, Caucasians; H, Hispanics.

**Table 5.** Plasma Measurements in Healthy Controls and Patients with Schizophrenia

Plasma Parameter	Controls	Schizophrenics	<i>p</i>
Clearance total activity (L/h)	15.7 ± 1.8	16.7 ± 3.0	.247
Fraction parent (%)	14 ± 7	12 ± 3	.077
Clearance parent [ <sup>123</sup> I]iomazenil (L/h)	116 ± 23	142 ± 36	.023
<i>f</i> <sub>1</sub> normalized	0.32 ± 0.05	0.32 ± 0.05	.697
Clearance free [ <sup>123</sup> I]iomazenil (L/h)	370 ± 86	441 ± 115	.058

Values are Mean ± SD; *p* is the probability value of between group differences by two tailed unpaired t-test.

est binding was observed in neocortical areas, parahippocampal gyrus, and cerebellum, and lower values in amygdala, hippocampus, striatum and thalamus. The variance of regional BDZ *V*<sub>T</sub> was not significantly different between the groups.

We observed no between group differences in regional BDZ *V*<sub>T</sub> between controls and schizophrenics in any of the regions examined (Table 6). Figure 1 shows the distributions of BDZ *V*<sub>T</sub> in left and right prefrontal region in both groups. In controls, laterality indices were significant for a right dominance (i.e., significantly lower than zero, one sample t-test) in the temporal cortex (*p* = .036), occipital cortex (*p* = .011), and striatum (*p* = .032). In schizophrenics, no region had a significant lateralization index. However, no significant between groups differences were observed in the laterality indices.

Results of the SPM analysis were in accordance with the region of interest analysis: no significant statistical excursions were detected by comparing the BDZ *V*<sub>T</sub> maps between patients and controls, either on the normalized or non normalized analyses.

No effect of age was observed on regional BDZ *V*<sub>T</sub> in controls or in schizophrenics, and there was no age \* diagnosis interaction. No regional BDZ *V*<sub>T</sub> differences were observed between the patients off (*n* = 5) or on neuroleptics (*n* = 11). No regional BDZ *V*<sub>T</sub> differences were observed between patients with (*n* = 4) or without (*n* = 12) significant tardive dyskinesias. Correlations were investigated between regional BDZ *V*<sub>T</sub> and positive and negative symptoms severity. BDZ *V*<sub>T</sub> in several regions was positively correlated with severity of positive symptoms: left amygdala, *p* = .018; right hippocampus, *p* = .0076; right striatum, *p* = .033; right thalamus, *p* = .0331. No regional BDZ *V*<sub>T</sub> was correlated with negative symptoms. Since a total of 40 correlations were performed (10 regions \* 2 sides \* 2 clinical clusters), none of the above mentioned correlations survived a Bonferroni correction for significance. To attempt to replicate the observation of Schröder et al., we tested the correlation between BPRS scores and prefrontal BDZ *V*<sub>T</sub>: these measured were not correlated (*p* = .63 and .33, for left and right prefrontal *V*<sub>T</sub>, respectively).

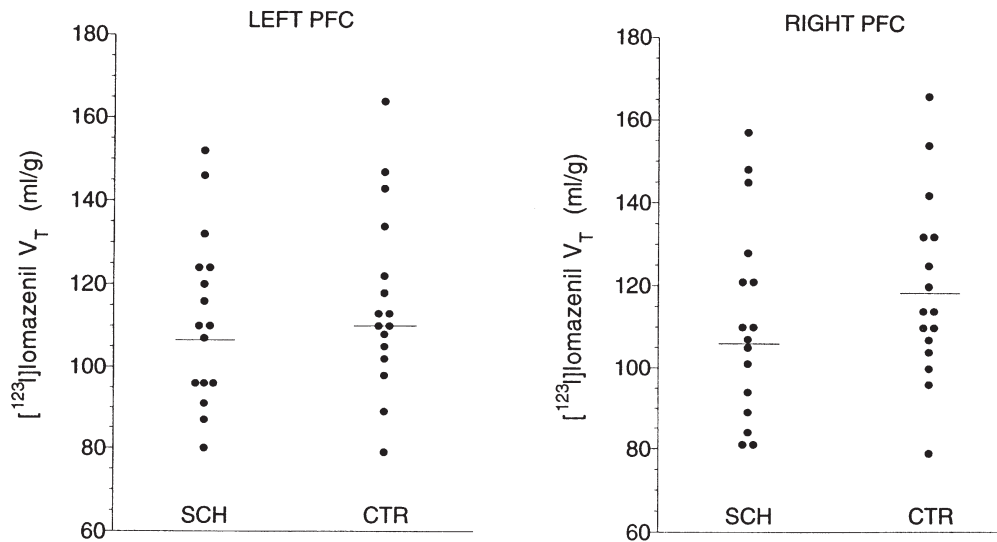
## DISCUSSION

This study failed to demonstrate any alterations in the density and regional distribution of BDZ receptors in patients with schizophrenia. A preliminary report of this study suggested an increased BDZ receptor availability in several regions of schizophrenic subjects compared to controls (*n* = 7 per group), a difference that reached significance in the occipital cortex (Abi-Dargham et al. 1994a). However, in this extended sample, no significant differences between schizophrenics

**Table 6.** Regional BDZ Distribution Volumes (*V*<sub>TFCOR</sub> ml/g) in Schizophrenic Patients and Controls

Region	Controls			Schizophrenics			Significance		
	Left Side	Right Side	AI	Left Side	Right Side	AI	<i>p</i> <sup>(1)</sup>	<i>p</i> <sup>(2)</sup>	<i>p</i> <sup>(3)</sup>
Cortical regions									
Frontal cortex	116 ± 22	119 ± 22	-0.02 ± 0.03	112 ± 21	111 ± 24	0.00 ± 0.04	.59	.32	.12
Temporal cortex	108 ± 23	111 ± 20	-0.02 ± 0.03*	105 ± 20	108 ± 19	-0.01 ± 0.05	.77	.66	.72
Parietal cortex	97 ± 19	99 ± 25	0.00 ± 0.06	98 ± 19	99 ± 19	0.00 ± 0.03	.83	.99	.97
Occipital cortex	112 ± 21	119 ± 24	-0.03 ± 0.04*	111 ± 22	115 ± 21	-0.02 ± 0.04	.85	.68	.70
Limbic Regions									
Cingulate cortex	112 ± 26	107 ± 18	0.01 ± 0.12	112 ± 26	108 ± 30	0.03 ± 0.03	.98	.95	.78
Amygdala	57 ± 21	61 ± 16	-0.04 ± 0.10	60 ± 14	63 ± 17	-0.02 ± 0.12	.64	.67	.56
Hippocampus	62 ± 20	65 ± 18	-0.03 ± 0.08	58 ± 14	65 ± 16	-0.06 ± 0.10	.56	.48	.97
Parahippocampal gyrus	91 ± 30	100 ± 24	-0.05 ± 0.12	91 ± 20	96 ± 27	-0.02 ± 0.11	.94	.66	.45
Striatum	57 ± 11	58 ± 15	-0.04 ± 0.07*	54 ± 13	56 ± 10	-0.02 ± 0.08	.47	.77	.34
Thalamus	61 ± 14	62 ± 15	0.01 ± 0.06	56 ± 14	57 ± 13	-0.02 ± 0.06	.27	.35	.75
Cerebellum		121 ± 20			120 ± 21			.81	

Values are Mean + SD; AI is the asymmetry index, calculated as (L - R)/(L + R); *p* is the probability value of between group difference by two tailed unpaired t test; *p*<sup>(1)</sup> is the probability of a regional difference comparing right regions; *p*<sup>(2)</sup> is the probability of a regional difference comparing left regions; *p*<sup>(3)</sup> is the probability of a difference in the asymmetry index.



**Figure 1.** Scatter plot of benzodiazepine receptors distribution volumes ( $V_T$ ) measured with [ $^{123}$ I]iomazenil SPECT in left and right prefrontal cortex (PFC) in 16 patients with schizophrenia (SCH) and 16 controls (CTR). No significant between-groups differences were detected.

and controls were observable. Furthermore, unlike previous in vivo imaging studies, we were unable to detect any correlations between clinical symptoms and regional BDZ receptors density.

This study was performed with the constant infusion/equilibrium analysis technique (Abi-Dargham et al. 1994b). As previously discussed, this method is easier to implement than the kinetic analysis of single bolus experiments, as it does not require arterial sampling, multiple plasma measurements and prolonged imaging time. Compared to semi-quantitative "ratio" methods following single bolus injection, the constant infusion method is more accurate, since the results are not influenced by possible between groups differences in peripheral clearance or regional blood flow.

While the reproducibility and accuracy of the method had been previously determined in baboons (Laruelle et al. 1994), we had not yet examined the performance of this method in humans. We previously reported the reproducibility and reliability of measurement of regional BDZ distribution volumes with [ $^{123}$ I]iomazenil in humans derived by kinetic analysis of single bolus experiments (6 pairs) (Abi-Dargham et al. 1995). In the present study, the reproducibility and reliability of outcome measures determined with the constant infusion were slightly inferior to the kinetic study. The variability (reliability) of  $V_T$  was  $13 \pm 1\%$  (0.66), compared to  $10 \pm 2\%$  (0.81) in the kinetic study, and the variability (reliability) of  $V_T$  was  $15 \pm 1\%$  (0.70), compared to  $13 \pm 2\%$  (0.61) in the kinetic study. The advantage in reproducibility and reliability of the single bolus/kinetic method over the constant infusion/equilibrium method is not surprising, since more data are acquired for the kinetic

method: 145 min of scanning time (versus 40 min for the equilibrium method) and 18 arterial samples (versus 3 venous samples for the equilibrium method). The slight gain in reproducibility of the kinetic method may not justify the considerable increase in labor, cost, and patient discomfort associated with this method. However, this observation suggests that acquiring data for longer period of time and measuring more blood samples could improve the reproducibility of the equilibrium method.

In this study, the reproducibility of  $f_1$  measurements were excellent, as it was in the kinetic study ( $3 \pm 3\%$  and  $6 \pm 5\%$ , respectively), yet the reliability was poor (0.54 in this study versus 0.46 in the kinetic study). This result confirms the previous observation that  $f_1$  measurements, however accurate, are of little significance compared to the small between-subjects variability observed in these test/retest studies. However, subjects included in these test/retested studies were young and healthy, and this conclusion may not apply to comparison of more heterogeneous groups. Therefore, in this clinical study, we decided to use  $V_T$  as outcome measure, because: 1) the use of  $V_T$  corrects for between subjects differences in the free fraction; and 2) the cost of this correction in terms of reproducibility/reliability is not apparent, since no significant differences were observed in the test/retest study between the reproducibility and reliability of  $V_T'$  and  $V_T$ .

The importance of including the plasma free fraction in the computation of the distribution volumes is illustrated by the following observation. In this study, we observed a trend toward decreasing  $f_1$  values with age in the schizophrenic group (decrease of 0.02% per de-



cade,  $p = .14$ ), but not in the control group. In the schizophrenic group, we observed a parallel trend toward a decrease in  $V_T'$  values with age, and this "age" effect on  $V_T'$  reached significance in the frontal, cingulate and cerebellar regions. Since BDZ receptor number is not affected by age in healthy subjects (Abi-Dargham et al. 1994b), an age effect on BDZ receptors in schizophrenia would have been a potentially interesting observation. In addition, one postmortem study had described such a correlation in prefrontal regions of schizophrenic brains (Kiuchi et al. 1989). However, in this particular sample of schizophrenic patients, less free [ $^{123}$ I]iomazenil was available for binding to BDZ receptors in older subjects compared to younger subjects. After correction of the volume of distribution by the free fraction (i.e. using  $V_T$  instead of  $V_T'$ ), the "age effect" disappeared. The important point is that the absence of correction for the free fraction might have induced artifactual results.

A similar observation illustrates the potential pitfalls of not measuring peripheral metabolism of [ $^{123}$ I]iomazenil. In this study, schizophrenics showed a faster metabolism than controls, which resulted in both lower plasma values and lower total [ $^{123}$ I]iomazenil regional uptake. Expressing the BDZ distribution volume relative to the total activity (metabolites and parent), i.e. using  $V_{T\text{ ACT}}$  as outcome measure, would have resulted in observing significantly lower "BDZ receptors" in schizophrenic patients in frontal, thalamic and cerebellar regions. Worse, expressing the regional uptake relative to the injected dose ( $V_{T\text{ INF}}$ ) would have resulted in observing significantly lower BDZ distribution volumes in frontal, temporal, occipital, cingulate, striatal, thalamic and cerebellar regions. These points illustrate the potential pitfalls of semi-quantitative approaches to in vivo neuroreceptor imaging.

In agreement with the study of Busatto et al. (1997), we found no differences in any regions in BDZ receptors between schizophrenics and controls. Busatto et al. (1997) reported several relationships between regional BDZ density and severity of positive and negative symptoms. The authors acknowledged that these correlations were exploratory at best, and that they did not survive Bonferroni corrections. The fact that these correlations were not observed in the present study, which included a similar number of subjects, and an improved quantitative method (equilibrium conditions, plasma measurement, and MRI based ROI analysis) confirmed that these correlations were indeed statistical artifacts. The same comment applies to the correlation between BPRS scores and frontal [ $^{123}$ I]iomazenil uptake reported by Schröder et al. (1997). Together, these studies indicate that in vivo BDZ receptor density, measured with SPECT [ $^{123}$ I]iomazenil, is not affected in schizophrenia, and is not related to symptomatology as previously claimed.

Evidently, this conclusion is only valid for the level of resolution obtained in vivo. The spatial resolution of

SPECT limits the accuracy of the measurement in small structures, such as the hippocampus and amygdala. At best, the resolution of SPECT is comparable to the resolution of homogenate binding studies. Careful review of postmortem homogenate binding literature does not provide consistent evidence for alteration of BDZ receptors in schizophrenia. Four postmortem investigations have examined the binding of the BDZ agonist [ $^3$ H]flunitrazepam in homogenate membranes of schizophrenic brains. Changes reported by Squires et al. (1993) (a 30–60% decrease in [ $^3$ H]flunitrazepam binding in homogenated membranes of hippocampus and cingulate cortex) should be detectable with SPECT, if present in this sample. The significance of the Squires study is hampered by the small number of subjects ( $n = 4$  per groups). In a larger sample (13 schizophrenics and 10 controls), Kiuchi et al. (1989) reported a 25% increase in most of the regions examined (which included frontal, temporal, hippocampus and putamen). In the medial frontal cortex, there was no overlap between the schizophrenic and control values. Again, an increase of this magnitude should be detectable with SPECT, and the reason for the discrepancy between this postmortem and the in vivo studies reported here and elsewhere is not obvious. The results of the present investigation agree with the postmortem studies of Reynolds and Stroud (1993) which included 15 schizophrenics and 13 controls, and failed to show group differences in [ $^3$ H]flunitrazepam  $B_{\text{max}}$  and  $K_D$  in the hippocampus, and of Pandey et al. (1997) who showed no changes in [ $^3$ H]flumazenil  $K_D$  or  $B_{\text{max}}$  in the prefrontal cortex of schizophrenic patients. Altogether, as one study showed an increase in density (Kiuchi et al. 1989), one showed a decrease (Squires et al. 1993), and three, in addition to this one, showed no change (Reynolds and Stroud 1993; Busatto et al., 1997; Pandey et al. 1997) it is reasonable to propose that no substantial abnormalities in BDZ density are present in schizophrenic patients. Our results are also consistent with the observation that mRNA expression of the main subunits of the adult GABA $_A$ /BDZ receptor complex is unaltered in prefrontal cortex in schizophrenia (Akbarian et al. 1995a). Obviously, these investigations cannot rule out potential alterations limited to specific layers of the cortex or subfields of the hippocampus, such as recently described (Benes et al. 1997).

Interestingly, Pandey et al. (1997) reported downregulation of BDZ receptors in the frontal cortex of schizophrenic patients who died while taking neuroleptic drugs compared to schizophrenic patients who were off drugs at time of death. In the present study, we failed to replicate these results, since no regional differences in BDZ  $V_T$  were observed between patients on or off neuroleptics. Thus our results do not support alteration of BDZ receptors density associated with long-term neuroleptic exposure, a conclusion consistent with

preclinical studies (Gavish et al. 1988; Giardino et al. 1991).

The lack of change in BDZ receptors is at variance with the observation of increased [<sup>3</sup>H]muscimol binding to GABA<sub>A</sub> receptors in schizophrenia described with homogenate binding in the left prefrontal cortex (increase of about 25%, number of schizophrenic cases, 19, Hanada et al. 1987) and with autoradiography in several layers of anterior cingulate ( $n = 6$ ; Benes et al. 1992), and hippocampus subfields ( $n = 8$ ; Benes et al. 1996). Observing little differences in BDZ binding on the same brain regions where marked differences in [<sup>3</sup>H]muscimol binding had been measured, Benes et al. (1997) proposed that schizophrenia might be associated with an uncoupling of GABA<sub>A</sub> and BDZ receptors. Since the pattern of expression of GABA<sub>A</sub>/BDZ receptors subunits mRNA is unchanged in schizophrenia (Akbarian et al. 1995a), the mechanism underlying this putative uncoupling remains to be elucidated. The development of an in vivo GABA<sub>A</sub> radiotracer would be useful to explore this issue further.

In conclusion, this study failed to detect any significant abnormalities of BDZ receptors density in a sample of 16 patients with schizophrenia compared to 16 healthy controls, and contradicts previous reports of an association between BDZ receptor density and symptomatic features of the illness. If a deficit in GABA function is implicated in the pathophysiology of schizophrenia, this deficit might not involve a substantial alteration in the expression of BDZ receptors. In vivo neuroreceptor studies of the GABA site on the GABA<sub>A</sub>/BDZ complex or magnetic resonance spectroscopy studies of GABA levels are needed to provide additional examination of the GABA deficit hypothesis of schizophrenia.

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