Vesicular Monoamine Transporter Concentrations in Bipolar Disorder Type I, Schizophrenia, and Healthy Subjects


**Background:** Previous analyses of vesicular monoamine transporter (VMAT2) binding in euthymic bipolar disorder type I (BDI) patients have shown increases of this presynaptic marker in the thalamus and ventral midbrain. To assess the diagnostic specificity of those findings, we compared VMAT2 concentrations between euthymic BDI patients, patients diagnosed with schizophrenia (SCH), and age-matched healthy volunteers.

**Methods:** Binding sites for VMAT2 were quantified with (+)-α-[¹¹C]DTBZ (dihydrotetrabenazine) and positron emission tomography. Fifteen euthymic BDI and 12 SCH patients and 15 group-matched healthy controls were studied. [¹¹C]DTBZ tracer transport and binding potentials were examined in the thalamus and ventral midbrain with factorial analyses of variance and post hoc Tukey’s honestly significantly different tests.

**Results:** Analysis of variance detected diagnosis effects in binding potentials in both brain regions. Binding of VMAT2 in the thalamus was higher in BDI patients than in control subjects and SCH patients. Conversely, ventral brainstem binding was nearly identical between BDI and SCH patients and were higher than in the control group.

**Conclusions:** The patterns of regional VMAT2 expression, and by extension, the concentration of monoaminergic synaptic terminals, differ between BDI, SCH, and a control group. These findings may relate to both similarities and differences in the presentation or clinical course of these syndromes and require further examination.


**Key Words:** Dopamine, serotonin, VMAT2, schizophrenia, bipolar disorder, positron emission tomography

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**Introduction**

Previous analyses on euthymic patients diagnosed with bipolar disorder type I have shown regional increases in a putative marker of monoaminergic synaptic density, the central vesicular monoamine transporter (VMAT2), relative to healthy control subjects. Two regions were different between patients and control subjects, the thalamus and ventral midbrain (Zubieta et al 2000). Both schizophrenia (SCH) and bipolar disorder type I (BDI) can present with similar features, namely, the presence of psychotic symptoms during active phases of the illness, but with distinct clinical courses and associated features (Kraepelin 1919, 1921). Because the vast majority of the subjects in our BDI sample had a prior history of mania with psychosis, it was of interest to examine whether similar patterns of VMAT2 binding would be observed in SCH. This would perhaps relate the observed increases in VMAT2 concentrations to a predisposition to the development of psychotic symptoms as opposed to more diagnostic-specific features of BDI. For this purpose, we compared the concentration of VMAT2 sites in the thalamus and ventral brainstem between a group of stable SCH patients, a sample of euthymic BDI patients, and healthy volunteers. Binding of VMAT2 was measured with (+)-α-[¹¹C]dihydrotetrabenazine (DTBZ) and positron emission tomography (PET) (Koeppe et al 1996; Lee et al 1996). The VMAT2 binding site is exclusively located in the membranes of presynaptic vesicles of monoaminergic neurons (Henry and Scherman 1989). It mediates the transport of monoamine neurotransmitters from the cytoplasm to their storage vesicles and regulates the quantal release of monoamines proportionally to its expression in transgenic animal models (Fon et al 1997). Measures of VMAT2 concentrations have been proposed as an index of presynaptic terminal integrity (Vander Borght et al 1995a). Unlike the plasma membrane reuptake transporters (e.g., dopamine transporter [DAT]), VMAT2 sites do not appear readily regulated by drugs affecting monoaminergic neurotransmission. Acute or prolonged (5 days to 2 weeks)
administration of dopamine agonists or antagonists, monoamine oxidase inhibitors, dopamine precursors (L-DOPA), DAT blockers, or antimuscarinic agents, have not been shown to modulate this site, whereas regulation of the dopamine D2 receptors and DAT were observable (Kilbourn et al 1996; Naudon et al 1994; Vander Borght et al 1995a). Similarly, the chronic self-administration of cocaine for a mean of 7 weeks did not alter the brain regional concentrations of this site in experimental animals (Wilson et al 1996). Studies in lesioned animals have also shown that the concentrations of VMAT2 sites are proportional to the density of monoaminergic terminal fields (Kilbourn et al 2000; Naudon et al 1992; Vander Borght et al 1995b). These properties have permitted the interpretation of VMAT2 binding sites measured with \(^{11}\text{C}\)DTBZ as an index of monoaminergic innervation in human neurodegenerative disorders. Striatal binding decreases with advancing age (approximately 0.5%–0.8% per year) and is reduced in various disorders affecting dopaminergic innervation (Frey et al 1996a; Gilman et al 1996). In this report we examined similarities and differences in the expression of the VMAT2 in BDI, SCH, and healthy control subjects.

**Methods and Materials**

**Subjects**

Fifteen euthymic patients diagnosed with bipolar disorder type I (9 men, 6 women, 39 ± 12 years old, 16 ± 2 years of education), and 12 patients diagnosed with schizophrenia (8 men, 4 women, 36 ± 11 years old, 13 ± 3 years of education) were studied. Fifteen healthy volunteers, matched by age to both groups (9 men, 6 women, 38 ± 11 years old, 16 ± 2 years of education) were recruited through advertisement. All volunteers underwent structured diagnostic interviews to confirm the clinical diagnoses in the patients and to rule out the presence of undiagnosed psychiatric illness in the control group (Structured Clinical Interview for DSM-IV Axis I Disorders: First et al 1995). Patients with BDI were studied during a prolonged asymptomatic phase, as previously described (Zubieta et al 2000). With the exception of one subject, all the patients in this group had a history of psychosis during manic states. All BDI patients were free of alcohol or substance abuse or dependence by DSM-IV criteria with the following exceptions: two BDI patients who met criteria for alcohol abuse only during manic episodes but had discontinued use of alcohol 6 and 15 years prior to the study; and two SCH patients who had prior diagnoses of alcohol abuse (including one later discovered to have used cocaine for approximately 4–6 months) which were in remission for more than 5 years.

After complete description of the study to the subjects, written informed consent was obtained. All procedures were approved by the University of Michigan Institutional Review Boards.

**Scanning Procedures**

Positron emission tomography imaging was performed on a Siemens/CTI (Knoxville) ECAT Exact-47 scanner in three-dimensional mode (septa retracted). A 12 min transmission scan was obtained immediately prior to PET emission acquisition for attenuation correction. Approximately 18 mCi (666 MBq) (+)-\(\text{-}\)\(^{11}\text{C}\)dihydrotetrabenazine (Jewett et al 1997) were administered intravenously per subject. Half of the dose was administered as a bolus over 1 min, and the remainder as a continuous infusion over the remainder 59 min of acquisition, to achieve tracer equilibrium between plasma and brain between 30 and 60 min post-administration (Koeppe et al 1997). Scanning was performed during 0–4 min as a continuous acquisition, followed by 3 × 10 min scans from 30 to 60 min. Data were reconstructed using a Hanning filter with a cutoff of 0.5 cycles/projection ray using both scatter and attenuation corrections. The final images were reconstructed into a 128 × 128 matrix with 2.25 mm\(^3\) size isotropic voxels, 60 planes, and a resolution of approximately 9 mm full-width at half maximum.

Blood samples were withdrawn using a radial artery catheter and utilized to construct the metabolite-corrected plasma input function, as previously described (Frey et al 1996b). Radioactive fiducial markers placed on the subject’s scalp were used to coregister the images and to correct for patient motion that may have occurred during the study (Koeppe et al 1997).

Two sets of parametric images were then calculated on a pixel-by-pixel basis using these dynamic scan data: (1) \(\text{DV}_{\text{tot(eq)}}\) or total brain tissue distribution volume of the radiotracer relative to plasma at equilibrium. This measure is obtained by dividing the concentration of \((+)-\alpha-\text{\[^{11}\text{C}\]DTBZ in brain tissue (measured with PET) by the metabolite-corrected arterial plasma averaged over the portion of the study after equilibrium conditions are achieved (30–60 min post-tracer administration). The \(\text{DV}_{\text{tot(eq)}}\) is independent from changes in the transport rate of the radiopharmaceutical (Koeppe et al 1997). It represents the sum of specific VMAT2 binding and nonsaturable (free ligand + nonspecific binding) \[^{11}\text{C}\]DTBZ levels, and is proportional to the distribution of VMAT2 binding sites (Frey et al 1996a); (2) \(K_p\), or plasma-to-tissue transport rate of the radiopharmaceutical, is then calculated by an autoradiographic method using the corresponding DV estimate for each pixel (Herscovitch et al 1983).

Images were anatomically standardized by alignment of the \(K_p\) images to the intercommissural line (Minoshima et al 1993), nonlinear warping to the Talairach and Tournoux atlas (Talairach and Tournoux 1988) stereotactic coordinates (Minoshima et al 1994) and applying the same transformation matrices to the
$DV_{\text{tot(eq)}}$ images (Frey et al 1996b). Square, three-by-three pixel regions-of-interest (ROI, 6.75 mm side) were placed on the $K_1$ images in three consecutive planes by an operator blind to diagnosis (PH) and confirmed by one of us (JKZ), also blindly. ROIs were placed using a predefined ROI template (one ROI each side) and the regional definitions and coordinates of the Talairach and Tournoux atlas, and then transferred to the $DV_{\text{tot(eq)}}$. Two regions were examined: thalamus and ventral brainstem. The middle ROI for each structure was centered by one of us (JKZ), also blindly. The occipital cortex was also sampled to provide an estimate of nonsaturable activity, as previously described (Frey et al 1996a) (4 ROIs each side). Occipital cortex (octx) values were then utilized to normalize $K_1$ values (normalized $K_1 = \frac{\text{ROI}}{\text{octx} K_1}$) to reduce interexperimental variability due to global scaling factors (i.e., variability in the plasma metabolite correction procedures, calibration factors for scanner or well counters, etc.). They were also utilized to obtain an estimate of normalized specific DTBZ binding ($DV_{\text{tot(eq)}}$ (binding potential) [11 C]DTBZ binding to vesicular monoamine transporter (VMAT2) sites in the same groups. Data represent the mean ± SD (error bars) of normalized $K_1$ and specific DTBZ binding (binding potential) to VMAT2 sites in a healthy comparison group (white bars), patients diagnosed with bipolar disorder I (patterned bars), and schizophrenia (black bars). *Significantly different from healthy control group. #Significantly different between bipolar disorder I and schizophrenia groups. Analysis of variance with post hoc Tukey’s honestly significantly different test, $p < .05$.

Results

No significant differences in age were noted between subject groups (unpaired, two-tailed $t$ tests, $p > .05$). Educational level was lower in SCH patients ($13 \pm 3$ years) than in BDI patients ($16 \pm 2$ years, $t(25) = 3.37$, $p < .01$) or healthy subjects ($16 \pm 2$ years, $t(25) = 3.15$, $p < .01$).

$[11 \text{ C}]$DTBZ Tracer Transport ($K_1$)

To assess whether differences in tracer transport were present (due to factors such as differences in regional cerebral blood flow or to differences in regional brain volumes), regional normalized $K_1$ rates were compared between groups. Analysis of variance identified a diagnosis effect in the thalamus [$F(2,38) = 4.86$, $p = .013$], with post hoc Tukey’s HSD showing higher normalized $K_1$ values in BDI patients compared to SCH but no significant differences between BDI or SCH and healthy subjects ($p < .05$). No significant effects of diagnosis on normalized $K_1$ rates were detected in the ventral brainstem [$F(2,38) = 1.02$, $p = .36$] (Figure 1A).

$[11 \text{ C}]$ DTBZ Binding to VMAT2 Sites

Regional distribution volume ratios at equilibrium ($DV_{\text{tot(eq)}}$), prior to the estimation of specific binding potentials were as follows: a) occipital cortex, control subjects $= 5.5 \pm 1.2$; BDI $= 5.6 \pm 1.1$; SCH $= 4.8 \pm 0.7$;
b) thalamus, control subjects = 6.4 ± 1.3; BDI = 6.7 ± 1.4; SCH = 5.5 ± 0.7; c) ventral brainstem, control subjects = 7.2 ± 1.7; BDI = 7.7 ± 1.7; SCH = 6.6 ± 1.2. Somewhat lower nonspecific and free distribution volumes in the occipital cortex were observed in the SCH sample, which did not reach statistical significance between groups [ANOVA, \( F(2,39) = 2.41, p > .1 \)]. Absolute DV_{tot(eq)} were also slightly lower in the SCH group for the remainder of the regions as well, perhaps reflecting globally decreased nonspecific and free distribution volumes or altered plasma protein or blood cell bindings in this group.

After calculation of specific BP values, ANOVA showed a significant effect of diagnosis in the thalamus [\( F(2,39) = 4.03, p = .025 \)]. Post hoc Tukey’s HSD demonstrated increases in BP in BDI patients compared to healthy control subjects and SCH patients (\( p < .05 \)) but no differences between control subjects and SCH patients. Mean values were nearly identical for the latter two groups (Figure 1B). Conversely, diagnosis effects were also noted in the ventral brainstem [\( F(2,39) = 4.50, p = .017 \)], but with significant increases in both BDI and SCH groups compared to control subjects (Tukey’s HSD, \( p < .05 \)) (Figure 1B).

**Discussion**

Prior data from our laboratory has shown that euthymic BDI patients with a prior history of mania with psychosis presented increases in VMAT2 binding in comparison with healthy volunteers in the thalamus and ventral brainstem (Zubieta et al. 2000). This was thought to reflect group differences in monoaminergic synaptic terminal fields (Vander Borght et al. 1995b). As a result, it was of interest to examine whether similar VMAT2 binding patterns would be present in SCH patients. Similar findings in the SCH group would relate the presence of VMAT2 anomalies to a predisposition for the development of psychotic symptoms and not specific diagnostic categories.

The data obtained points to both similarities and differences in VMAT2 binding patterns in SCH and BDI. Both patient groups showed similar mean increases in binding in the ventral brainstem compared with the control group: 37% in SCH and 32% in BDI; however, the higher thalamic binding observed in BDI relative to control subjects (mean difference 32%) was not noted in the SCH sample.

The above-noted differences in regional VMAT2 concentrations were not coupled with parallel changes in regional tracer transport, a measure proportional to blood flow. In the thalamus, \( K_1 \) values were slightly lower in SCH than in the healthy comparison group and slightly higher in BDI than in control subjects. Significant differences were only encountered between SCH and BDI groups, with higher values in the latter. Although differences in tracer transport do not affect the binding potential measures obtained at equilibrium, they could represent differences in thalamic regional blood flow, or in the volume of this structure, or both, between BDI and SCH patients. No significant group differences in the transport rate of the radiopharmaceutical were noted in the ventral brainstem. Reductions in the volume of the thalamus have been documented in SCH in some, but not all studies (Gur et al. 1998; Hazlett et al. 1999; McCarley et al. 1999). Conversely, volumetric changes in this structure have not been clearly demonstrated in BDI, even using much higher resolution techniques (Strakowsky et al. 1993, 1999). The possible contribution of differences in regional brain volumes to the findings presented in the thalamus would require further investigation; however, we accounted for differences in global reductions in the nonspecific and free distribution volume of the radiotracer by using occipital cortex DV_{tot(eq)} as an estimate of nonsaturable activity, reducing possible nonspecific volumetric contributions to the data. Also, the fact that the changes in volume so far observed with magnetic resonance techniques are relatively small in comparison to the effects noted in this article indicates that they are not likely to entirely account for the findings reported. The size of the regions of interest applied to the image data (approximately one full width-half maximum of their resolution and centered over relatively large anatomical regions) also made the measures obtained less susceptible to nonspecific volumetric differences between groups (Links et al. 1996).

\[^{11}C\]DTBZ is a selective marker for VMAT2 (Lee et al. 1996). This protein, located in the membrane of the synaptic vesicles (Henry and Scherman 1989), mediates the transport of the monoamines from the cytoplasm, into their presynaptic storage sites and regulates the release of monoamines from synaptic terminals (Reimer et al. 1998). Overexpression of the VMAT2 in monoaminergic cells is associated with increases in the quantal release of these neurotransmitters, whereas underexpression induces opposite effects (Fon et al. 1997). We selected to examine VMAT2 binding sites as a measure related to monoaminergic synaptic concentration or, alternatively, their functional capacity (Frey et al. 1996a; Reimer et al. 1998). These sites do not appear modulated by drugs known to regulate monoaminergic neurotransmission, such as various amine reuptake blockers, monoamine oxidase inhibitors, or antipsychotics. This is a desirable property in the study of illnesses that require continued medication administration (Krejci et al. 1993; Naudon et al. 1994; Vander Borght et al. 1995 Wilson et al. 1996). However, a
The disadvantage inherent to the use of the VMAT2 site as a marker of monoaminergic synaptic concentration is its nonselectivity for the various monoamines (Hoffman et al. 1998).

We observed similar increases in VMAT2 binding in the ventral brainstem of patients diagnosed with BDI and SCH, with respect to control values. The brainstem area contains the cell bodies and local projections of serotonergic (5HT) (raphe nuclei), and dopaminergic (DA) (retroorbital, substantia nigra, and ventral tegmental area) neurons. Its ventral region is rich in DA cells (regions A8, A9, and A10), although it also receives dense input from 5HT cells located in the raphe nuclei (Azmitia and Whitaker-Azmitia 1991). Caudally, the locus coeruleus is also the main source of forebrain noradrenergic innervation. It should be noted, however, that due to the relatively poor resolution of PET cameras, the values obtained from regions in close proximity, such as the ventral and dorsal brainstem, are likely to be contaminated by spillover of activity from one to the other. Therefore, the separation between ventral and dorsal brainstem should be taken with caution, and mostly as an approximation to binding values in those specific brain regions.

Most of the data acquired with in vivo imaging techniques has addressed the relative contribution of serotonergic or dopaminergic synaptic markers in this region. Prior studies with the nonselective serotonin-dopamine membrane transporter (SERT-DAT) ligand [123I]β-CIT and single photon emission computed tomography have shown that brainstem [123I]β-CIT labeling in humans and nonhuman primates is displaceable with SERT, but not DAT blockers (Laruelle et al. 1993; Pirker et al. 1995). This suggests that the majority of presynaptic terminals in this brain area are serotonergic, and it is therefore likely that ventral [11C]DTBZ uptake predominantly reflects 5HT terminal projections. In this regard, it has been suggested that 5HT input into brainstem DA cells is capable of modulating dopaminergic terminal development and function (Bolte Taylor et al. 1998; Di Mascio and Esposito 1997; Gongora-Alfaro et al. 1997; Ugedo et al. 1989). In turn, a dysfunction of dopaminergic neurotransmission has long been postulated to underlie some of the symptomatology encountered in schizophrenia and psychotic manic states (Breier et al. 1997; Hietala et al. 1999; Laruelle 1998; Pearlson et al. 1995; Wong et al. 1986). The possible contribution of noradrenergic terminals to the findings reported is unclear at this time, and its study will require the development of specific radiotracers labeling noradrenergic transporters or receptor sites, which are not available at this time.

The increases in VMAT2 concentrations observed in the thalamus of BDI patients compared to the SCH or control groups are again not likely due to changes in DA terminal fields. This brain region contains a high density of 5HT and noradrenergic terminals but negligible DA innervation in humans (Oke et al. 1997). In this regard, increased indexes of noradrenergic turnover in the thalamus and cortical regions have been observed in a postmortem study of BD patients (Young et al. 1994). Cerebrospinal fluid levels of 3-methoxy-4-hydroxyphenylglycol, the principal metabolite of noradrenaline, have also been observed increased in BD patients during manic phases (Swann et al. 1983), and in patients diagnosed with major depression and bipolar disorder during depressed states (Redmond et al. 1986). Resolving the specific neurochemical network or networks involved in the increases observed would require the development of specific radioligands labeling other transporters or receptors involved in serotonin or noradrenergic responses.

In summary, we observe both similarities and differences in regional VMAT2 binding (and presumably, monoaminergic synaptic density) between stable SCH and euthyemic BDI patients. Increases were noted in the ventral brainstem of both patient groups compared to control subjects, possibly reflecting higher levels of 5HT terminal innervation, or increased density of 5HT synaptic vesicles per synaptic contact in this area (Laruelle et al. 1993; Oke et al. 1997; Pirker et al. 1995). If this was the case, increased 5HT input could reflect anomalies in 5HT to DA regulation common to both BD and SCH. Because all the BD patients included in the sample except for one had a history of mania with psychosis, the findings may be related to a predisposition to the development of psychotic symptoms in these populations. Conversely, mean thalamic VMAT2 concentrations were nearly identical between SCH patients and healthy control subjects and increased in BD. The contribution of 5HT and noradrenergic terminals to this finding, and their relationship to the clinical features of BD, will require further studies. This preliminary report then justifies the further examination of monoaminergic synaptic projections and monoaminergic presynaptic markers in both BDI and SCH, and their possible relationships with clinical features of these illnesses.

J-KZ was supported by the General Clinical Research Center at the University of Michigan Grant No. M01RR00042 from the National Center for Research Resources, the Mental Illness Research Association’s Arthur Forrest Tull II Research Fund, and the National Association for Research in Schizophrenia and Depression. KAF was supported by Grant No. 5P50NS15655 from the National Institute of Neurological Disorders and Stroke.

The authors acknowledge our PET nuclear medicine technologists, Jill M. Rothley, Edward J. McKenna, Andrew R. Weeden, and Todd M. Hauser, for their dedication and work that made this study possible.
References


Di Mascio M, Esposito E (1997): The degree of inhibition of dopaminergic neurons in the ventral tegmental area is induced by selective serotonin reuptake inhibitors is a function of the density-power-spectrum of the interspike interval. Neuroscience 79:957–961.


treatments with haloperidol or bromocriptine do not alter the density of the monoamine vesicular transporter in the substantia nigra. *Neurosci Lett* 173:1–4.


