SYNAPSE 55:164–175 (2005)

Effects of Reduced Endogenous 5-HT on the In Vivo Binding of the Serotonin Transporter Radioligand ^{11}C-DASB in Healthy Humans

PETER S. TALBOT,[1*] W. GORDON FRANKLE,[1] DAH-REN HWANG,[1] YIYUN HUANG,[1,2] RAYMOND F. SUCKOW,[3] MARK SLIFSTEIN,[1] ANISSA ABI-DARGHAM,[1] AND MARC LARUELLE[1,2]

[1]*Department of Psychiatry, Columbia University College of Physicians and Surgeons and the New York State Psychiatric Institute, New York, New York 10032*

[2]*Department of Radiology, Columbia University College of Physicians and Surgeons and the New York State Psychiatric Institute, New York, New York 10032*

[3]*Analytical Psychopharmacology Laboratory, New York State Psychiatric Institute, New York, New York 10032*

KEY WORDS positron emission tomography; rapid tryptophan depletion; ^{11}C-DASB

ABSTRACT Although abnormal serotonin (5-HT) function is implicated in a range of mental disorders, there is currently no method to directly assess 5-HT synaptic levels in the living human brain. The in vivo binding of some dopamine (DA) radioligands such as ^{11}C-raclopride is affected by fluctuations in endogenous DA, thus providing an indirect measure of DA presynaptic activity. Attempts to identify a serotonergic radiotracer with similar properties have proved unsuccessful. Here, we investigated in humans the effects of reduced synaptic 5-HT on the in vivo binding of the 5-HT transporter (SERT) radioligand ^{11}C-DASB, using Positron Emission Tomography (PET) and the rapid tryptophan depletion (RTD) technique. Eight (8) subjects (5M, 3F) were scanned with ^{11}C-DASB under control and reduced endogenous 5-HT conditions, in a within-subject, double-blind, counterbalanced, crossover design. Regional distribution volumes (V_T) were calculated using kinetic modeling and metabolite-corrected arterial input function. ^{11}C-DASB specific binding was estimated as binding potential (BP) and specific to nonspecific equilibrium partition coefficient (V''_3), using the cerebellum as reference region. RTD caused small but significant mean reductions in ^{11}C-DASB V_T (-6.1%) and BP (-4.5%) across brain regions, probably explained by a concomitant reduction in ^{11}C-DASB plasma free fraction (f_1) of similar magnitude. No significant change in ^{11}C-DASB V''_3 was observed between control and reduced 5-HT conditions. Nor was there a significant relationship between the magnitude of tryptophan depletion and change in BP and V''_3 across individual subjects. These results suggest that ^{11}C-DASB in vivo binding is not affected by reductions in endogenous 5-HT. **Synapse 55:164–175, 2005.**

INTRODUCTION

Imaging brain serotonin (5-hydroxytryptamine; 5-HT) receptors is of interest because of their potential role in the pathophysiology and treatment of a range of psychiatric and behavioral disorders, including depression, impulsive aggression, early onset alcoholism, schizophrenia, obsessive-compulsive disorder, autism, gambling, and anxiety disorders. 5-HT receptors that can currently be imaged in humans using Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT) are the 5-HT_{1A} and 5-HT_{2A} receptors and the 5-HT transporter (SERT). Radiotracers for these receptors have been classically used to measure regional receptor densities and therapeutic drug occupancy in a number of the above clinical disorders (for review see Talbot and Laruelle, 2002). However, there is considerable current interest in the possibility that some serotonergic radioligands may provide an indirect measurement of

Contract grant sponsor: Public Health Serv Contract grant numbers: 1 R21MH/DA66505-01, 1-K02-MH01603-01t grant sponsor: Lieber Center for Schizophrenia Research at Columbia University.

*Correspondence to: Peter S. Talbot, MD, Division of Functional Brain Mapping, New York State Psychiatric Institute, Unit 31, 1051 Riverside Drive, New York, NY 10032. E-mail: pst2001@columbia.edu

Received 18 August 2004; Accepted 10 November 2004

DOI 10.1002/syn.20105

Published online in Wiley InterScience (www.interscience.wiley.com).

changes in the synaptic concentration of endogenous 5-HT, based on the principle that the radioligands will compete with 5-HT for receptor binding. This phenomenon of endogenous competition has been well characterized for the binding of certain radioligands to the dopamine (DA) D_2 receptor (for review see Laruelle, 2000). Thus, experimentally increased synaptic DA concentration results in reduced binding of ^{11}C-raclopride, ^{18}F-fallypride, and ^{123}I-IBZM, while synaptic DA depletion results in increased radiotracer binding. This technique has led to fundamental insights in a number of psychiatric disorders in which synaptic DA is pathologically disordered, particularly schizophrenia and substance abuse.

However, efforts to identify a serotonergic radiotracer vulnerable to endogenous competition in humans have so far proved less successful. To date, 5-HT_{1A} receptor binding has been most extensively investigated, using the high-affinity radioligand ^{11}C-WAY100635 and the moderate-affinity radioligand ^{18}F-MPPF. In mice and rats, radiolabeled WAY100635 binding is not consistently altered in 5-HT_{1A} receptor-rich regions by large increases and decreases in endogenous 5-HT, suggesting that a useful alteration in ^{11}C-WAY100635 binding may require a change in endogenous 5-HT concentration greater than can be routinely achieved (Hume et al., 2001; Maeda et al., 2001; Rice et al., 2001). This is supported by results in healthy humans showing that ^{11}C-WAY100635 binding is not altered by the reduced brain 5-HT that results from the technique of rapid tryptophan depletion (RTD) (Rabiner et al., 2002). For ^{18}F-MPPF, studies in rats proved promising, with significant alterations of radiotracer binding in hippocampus following experimentally increased and depleted 5-HT (Zimmer et al., 2002, 2003). However, binding was unchanged in healthy humans following alterations to brain 5-HT levels by tryptophan (Trp) infusion and depletion (De Haes et al., 2002), again suggesting that the increases in 5-HT levels needed to produce a measurable effect on ^{18}F-MPPF binding would be significantly greater than that possible with Trp manipulation. For 5-HT_{2A} receptor binding, fenfluramine-stimulated 5-HT fails to displace ^{11}C-MDL100907 in rats (Hirani et al., 2003), ^{3}H-N-methylspiperone in mouse brain (Rice et al., 2001), and ^{18}F-deuteroaltanserin in baboons (Staley et al., 2001).

Recently, Wilson and co-workers introduced ^{11}C-3-amino-4-[2-[(dimethylamino)methyl]phenylthio)-benzonitrile (^{11}C-DASB), a new PET radiotracer to image SERT (Ginovart et al., 2001; Houle et al., 2000; Wilson et al., 2000, 2002; Wilson and Houle, 1999). Studies in baboons and humans have shown that ^{11}C-DASB provides significant improvement over the previously developed SERT tracer ^{11}C-McN5652 (Suehiro et al., 1993), resulting from its higher specific to non-specific binding ratios, and measurable plasma free fraction (Frankle et al., 2004; Huang et al., 2002; Szabo et al., 2002). In addition, advances have been made in the understanding of the cellular processes regulating transmitter-radioligand interactions in vivo (Laruelle and Huang, 2001). In the case of neuroreceptors, both binding competition and receptor trafficking may be involved in the alterations in receptor availability measured by PET and SPECT following manipulation of endogenous neurotransmitter levels. For neurotransporters, details of differences and interactions between the binding of endogenous substrate and exogenous ligands are not fully characterized. However, animal in vitro and in vivo data suggest that SERT subcellular distribution is affected by levels of extracellular 5-HT (Blakely et al., 1998; Zahniser and Doolen, 2001). Thus, the investigation of the impact of endogenous 5-HT fluctuations on ^{11}C-DASB in vivo binding is warranted.

The rapid (or acute) tryptophan depletion paradigm (RTD) is a widely used dietary means of transiently and safely reducing brain 5-HT in humans (Moore et al., 2000). It is accomplished by the ingestion of an amino acid mixture that lacks Trp, the amino acid precursor of 5-HT. It results in reduced plasma and cerebrospinal fluid (CSF) Trp and CSF 5-HIAA over the subsequent several hours, reaching the lowest levels of plasma Trp at 5–6 hours and of CSF Trp at 8 hours post-ingestion (Carpenter et al., 1998; Klaassen et al., 1999; Williams et al., 1999). Under these conditions, reduced basal and stimulated brain 5-HT release have been confirmed in animals (Bel and Artigas, 1996; Gartside et al., 1992; Stancampiano et al., 1997). Brain 5-HT synthesis is dependent on the continued supply of dietary Trp across the blood-brain barrier (BBB). The reduction in brain 5-HT following RTD is thought to be a consequence of the relative inability of low levels of Trp to enter the CNS during the catabolic drive stimulated by the bolus of amino acids. This drive stimulates protein synthesis and the rapid removal of amino acids (including Trp) from the circulation into the tissues. Entry of Trp into the CNS is controlled by the ratio of the concentration of plasma-free Trp (i.e., the fraction unbound to plasma proteins) to that of the other large neutral amino acids, (LNAAs) tyrosine (Tyr), phenylalanine (Phe), leucine (Leu), isoleucine (Ile), and valine (Val) (Trp/ ΣLNAA ratio), that compete with Trp for the same transport mechanism across the BBB and the neuronal membranes (Bender, 1985). Thus, following a Trp-free amino acid mixture, the pre-existing free Trp competes unsuccessfully for transport into the CNS with the high level of recently ingested other LNAAs.

We present here the results of an investigation of the effects of reduced synaptic 5-HT on SERT availability, as measured by ^{11}C-DASB and PET in healthy humans, using RTD to reduce 5-HT. In line with the mechanism of receptor trafficking described above, the hypothesis was that reduced 5-HT would lead to SERT

internalization and, therefore, decreased ^{11}C-DASB-specific binding.

MATERIALS AND METHODS

Human subjects

The study was approved by the Institutional Review Boards of the New York State Psychiatric Institute and Columbia Presbyterian Medical Center. Eight (8) paid healthy volunteers participated in this study (age 30 ± 5 years, range 23 to 38, with these and subsequent values given as mean ± SD, 5 males and 3 females). The absence of medical, neurological, and psychiatric history (including alcohol and drug abuse) was assessed by history, review of systems, physical examination, routine blood tests, urine toxicology, and EKG. Because RTD can lead to a transient deterioration of mood in vulnerable populations, subjects were excluded who had current evidence of depressed mood as indicated by a score of 14 or greater on the Beck Depression Inventory (BDI) (Beck et al., 1961), or a family history of depression in a first-degree relative. In order to reduce confounding effects of hormonal status on 5-HT neurotransmission, female subjects were studied during the first 10 days of their menstrual cycle (early-mid follicular phase), and subjects using hormonal contraception were excluded. Pregnancy was excluded by serum pregnancy test at screening and on each day of scanning. All subjects provided written informed consent after receiving an explanation of the study.

Study design

Subjects participated in a double blind, within-subject, counterbalanced study of the effects of RTD on ^{11}C-DASB binding. Subjects were studied on 2 days separated by 16 ± 12 (range 6 to 39) days, and were instructed to abstain from drinks containing alcohol for 48 hours prior to each study day, to observe a low-protein diet on each preceding evening, and to fast from midnight prior to each study day. Subjects arrived at the study center 6–9 hours before their scheduled scanning time, and remained fasting until the end of each study day, although water was allowed and provided at all times.

After arrival, subjects rated their mood by completing the BDI and by marking the point that best indicated their subjective mood on a 100-mm visual analogue mood scale (VAMS) with "Happy" at one end and "Sad" at the other (Folstein and Luria, 1973). Following a venous blood draw (14 ml) for baseline plasma amino acid assay (see below), subjects were given a nutritionally balanced amino acid mixture to drink, either with (T+) or without (T−) Trp in it. Subjects drank the T+ mixture on the control day and the T− mixture on the RTD day. Investigators and subjects were blinded to the nature of the amino acid mixture on each occasion by the use of pre-packaged, coded mixtures. For male subjects, both mixtures contained 104.5 g of amino acids. The T+ mixture contained 16 amino acids in the following composition: L-alanine, 5.3 g; L-arginine, 4.7 g; L-cystine, 2.6 g; glycine, 3.1 g; L-histidine, 3.1 g; L-isoleucine, 7.7 g; L-leucine, 12.9 g; L-lysine, 8.4 g; L-methionine, 2.9 g; L-phenylalanine, 5.5 g; L-proline, 11.7 g; L-serine, 6.6 g; L-tyrosine, 6.6 g; L-valine, 8.5 g; L-threonine, 6.2 g; and L-tryptophan, 2.2 g. The T- mixture was identical except that the L-tryptophan was replaced by small amounts of dextrose, maltose, maltotriose, and higher saccharides to maintain a similar energy and nitrogen content. As females are known to experience a higher incidence of nausea following the amino acid mixtures typically used in RTD methodology, female subjects were given 83.5 g of the same mixtures. The drinks were made up in 250 ml of bottled mineral water. A citrus flavoring was added to improve palatability. Amino acid mixtures and flavoring were supplied by SHS International Ltd, Liverpool, UK.

On both study days, PET scans were scheduled to start 5–8 hours after the ingestion of the amino acid mixture. Across both study days and the 8 subjects, the order of administration of the RTD (T−) and control (T+) mixtures was fully counterbalanced. At 6.5 hours post-amino acid mixture, blood (14 ml) was again drawn for repeat plasma amino acid assay and subjects were re-scored on their subjective mood on the Happy-Sad VAMS. At the end of the scan, subjects were provided with a substantial meal and were returned home by taxi. The meal was chosen to contain significant amounts of Trp so that on the RTD day, serum Trp levels would return to baseline levels as quickly as possible.

At the end of the second scan day, subjects were discharged from the study. They received a single follow-up phone contact within the subsequent 7 days to enquire of any adverse effects of the study.

Plasma amino acid assays

On both study days, blood samples were taken before and 6.5 hours after ingestion of the amino acid mixture for measurement of total and free plasma Trp concentrations, and total plasma concentrations of the LNAAs Tyr, Val, Ile, Leu, and Phe. Blood (14 ml) was drawn into tubes containing ethylenediamine tetraacetic acid (EDTA) anticoagulant and immediately centrifuged for 10 min at 3,500 rpm. Plasma was then transferred by pipette to plain cryotubes and stored without delay at −40°C to await batch analysis.

Plasma amino acids were determined using a liquid chromatographic procedure (Hariharan et al., 1993) using norleucine as the internal standard. Plasma (0.25 mL) was deproteinized and extracted prior to derivatization with PITC reagent to enhance separation and detection at 254 nm. Calibration standards that included the expected concentration range for this study preceded each batch of samples as well as a set of

quality controls to validate each day's analyses. Interassay variability for all the amino acids measured did not exceed 14% (n = 25 days).

Total plasma Trp was measured using a validated unpublished liquid chromatographic procedure using native fluorescence of Trp for detection. An internal standard 5-methyltryptophan was added to the plasma sample (0.25 mL) followed by deproteinization and centrifugation. An aliquot of the supernatant was injected on column. Using a phosphate buffer (pH = 4.7) and acetonitrile as the mobile phase with a reversed phase ODS column (15 × 0.39 cm, 4 μ Novapak, Waters Corp.), Trp, and the internal standard eluted in less than 12 minutes. Fluorescence detection was optimized using an excitation wavelength at 290 nm and analyzed at 340 nm. Interassay variability of plasma tryptophan did not exceed 5.1% for the low-quality controls, and 7.2% for the high-quality controls (n = 22 days).

Unbound (free) Trp was determined using a 1-mL sample of plasma pipetted into a Centrifree Mircopartition tube and centrifuging at 3,000 rpm for 10 minutes. Twenty microliters (20 μL) of the resulting sample filtrate was injected under the same chromatographic conditions described above. A calibration curve was included with each day's run using the external standard method. The Lower Limit of Quantitation (LLOQ) for the LNAAs, total Trp, and free Trp was 25, 5, and 0.2 nmol/ml, respectively.

Radiochemistry

The standard DASB and precursor desmethyl DASB were obtained from the NIMH Chemical Synthesis and Drug Supply Program. Preparation of ^{11}C-DASB followed the literature procedure (Wilson et al., 2000), with some modifications as previously described by our group (Frankle et al., 2004).

PET protocol

PET imaging was performed with the ECAT EXACT HR+ (Siemens/CTI, Knoxville, TN) (63 slices covering an axial field of view of 15.5 cm, axial sampling of 3.46 mm, 3D mode in plane, and axial resolution of 4.4 and 4.1 mm full width half-maximum at the center of the field of view, respectively). An arterial catheter was inserted in the radial artery after completion of the Allen test and infiltration of the skin with 1% lidocaine. A venous catheter was inserted in a forearm vein on the opposite side. Head movement minimization was achieved with a polyurethane head immobilization system (Soule Medical, Lutz, FL) (Mawlawi et al., 1999). A 10-min transmission scan was obtained prior to radiotracer injection. ^{11}C-DASB was injected i.v. over 45 seconds. Emission data were collected in 3D mode for 100 min as 19 successive frames of increasing duration (3 × 20 s, 3 × 1 min, 3 × 2 min, 2 × 5 min, 8 × 10 min).

Input function measurement

Following radiotracer injection, arterial samples were collected every 10 seconds for the first 2 minutes and every 20 seconds from 2 to 4 minutes using an automated blood sampling system, and drawn manually thereafter at various intervals to a total of 30 samples. Selected samples (collected at 2, 16, 30, 50, 70, and 90 minutes) were further processed by high-pressure liquid chromatography (HPLC) to measure the fraction of plasma activity representing unmetabolized parent compound (Frankle et al., 2004). A biexponential function was fitted to the 6 measured unmetabolized fractions which was then used to interpolate values between the measurements. The smallest exponential of the unmetabolized fraction curve, λ_{par}, was constrained to the difference between λ_{cer}, the terminal rate of washout of cerebellar activity, and λ_{tot}, the smallest elimination rate constant of the total plasma activity (Abi-Dargham et al., 1999).

The input function was calculated as the product of total counts and interpolated unmetabolized fraction at each time point. The measured input function values ($C_a(t)$, mCi/mL) were fitted to a sum of three exponentials from the time of peak plasma activity, and the fitted values were used as the input to the kinetic analysis. The clearance of the parent compound (Cl, L/h) was calculated as the ratio of the injected dose to the area under the curve of the input function (Abi-Dargham et al., 1994). For the determination of the plasma-free fraction (f_1), triplicate 200-μL aliquots of plasma collected prior to injection were mixed with the radiotracer, pipetted into ultrafiltration units (Amicon Centrifree; Millipore, Bedford, MA), and centrifuged at room temperature (20 minutes at 4,000 rpm). At the end of centrifugation, the plasma and ultrafiltrate activities were counted, and f_1 was calculated as the ratio of activity in the ultrafiltrate to total activity (Gandelman et al., 1994). Triplicate aliquots of saline solution mixed with the radiotracer were also processed, to determine the filter retention of the free tracer.

MRI acquisition and segmentation procedures

For each subject, a 3-dimensional SPGR (Spoiled Gradient Recalled Acquisition in the Steady State) MRI scan was acquired on a GE 1.5 T Signa Advantage system, as previously described (Abi-Dargham et al., 2002). MRI Segmentation was performed within MEDx (Sensor Systems, Inc., Sterling, VA), with original subroutines implemented in MATLAB (The Math Works, Inc.; Natick, MA). Steps for MRI segmentation included correction for field inhomogeneities, fitting of the intensity distribution to a sum of 3 Gaussian functions, voxel classification, and post filtering (Abi-Dargham et al., 2000).

Image analysis

Images were reconstructed to a 128 × 128 matrix (pixel size of 2.5 × 2.5 mm^2). Reconstruction was performed with attenuation correction using the transmission data and a Shepp 0.5 filter (cutoff 0.5 cycles/projection ray). Reconstructed image files were then processed with the image analysis software MEDx (Sensor Systems, Inc.). If indicated by visual inspection, frames were realigned to a frame of reference, using a least-squares algorithm for within-modality coregistration (automated image registration, AIR) (Woods et al., 1992). The results of the frame-to-frame realignment were checked again visually. Following frame-to-frame registration, all frames were summed to one data set, which was coregistered to the MRI data set using AIR (Woods et al., 1992). The spatial transformation derived from the summed PET registration procedure was then applied to each individual frame. Thus, each PET frame was resampled in the coronal plane to a voxel volume of 1.5 × 0.9 × 0.9 mm^3.

Regions of interest (ROIs) and region of reference (cerebellum; CER) boundaries were drawn on the MRI according to criteria derived from brain atlases (Duvernoy, 1991; Talairach and Tournoux, 1988) and published reports (Kates et al., 1997; Killiany et al., 1997; Mawlawi et al., 2001; Pani et al., 1990). A segmentation-based method was used for the neocortical regions, and a direct identification method was used for the subcortical regions (Abi-Dargham et al., 2002). For bilateral regions, right and left values were averaged. The contribution of plasma total activity to the regional activity was calculated assuming a 5% blood volume in the ROIs (Mintun et al., 1984) and tissue activities were calculated as the total regional activities minus the plasma contribution.

Regions were considered to have a high enough SERT density to provide a reliable specific binding signal for analysis if mean V''_3 was ≥0.5 (Laruelle et al., 2003). Analysis was, therefore, restricted to the midbrain (MID, encompassing SERT-dense structures such as the raphe nuclei, substantia nigra, locus ceruleus, ventral tegmental area, and superior and inferior colliculi), thalamus (THA), caudate nucleus (CAU), putamen (PUT), ventral striatum (VST), amygdala (AMY), and entorhinal cortex (ENT).

Derivation of distribution volumes

Derivation of ^{11}C-DASB regional tissue distribution volumes (V_T, mL/g) was performed with kinetic modeling using the arterial input function and a three-compartment model (i.e., two-tissue-compartment model, 2TCM). Data from our laboratory demonstrated no clear advantage in the choice of analytic method, as all methods examined gave similar values across all regions. Although both a 1TCM and 2TCM provided virtually identical values of all outcome measures, examination of the fits indicated that the higher order model may provide a better fit in some studies (Frankle et al., 2003). V_T, which is equal to the ratio of tissue to plasma parent activity at equilibrium, was derived as:

$$V_T = \frac{K_1}{k_2}\left(1 + \frac{k_3}{k_4}\right)$$

where K_1 and k_2 are the rate constants governing the transfer of the ligand in and out of the nondisplaceable compartment (free and nonspecific binding), while k_3 (min^{-1}) and k_4 (min^{-1}) describe the rate of association and dissociation to and from the receptors, respectively (Koeppe et al., 1991; Laruelle et al., 1994a). Kinetic parameters were derived by applying sequential quadratic programming with bound (non-negativity) constraint, implemented in MATLAB (The Math Works, Inc.). The constraint was used since we found, as previously reported by others (Ginovart et al., 2001), that an unconstrained 2TCM failed to reliably converge. Given the unequal sampling over time (increasing frame acquisition time from the beginning to the end of the study), the least-squares minimization procedure was weighted by the frame acquisition time.

Derivation of SERT parameters

Derivation of SERT parameters was based upon the following assumptions: (1) because of the negligible density of SERT in the cerebellum (Backstrom et al., 1989; Laruelle et al., 1988; Plenge et al., 1990), cerebellum V_T was assumed to be representative of equilibrium nonspecific binding; (2) the nonspecific binding did not vary significantly between regions.

Two measures of SERT availability were calculated. The binding potential (BP, mL/g) was derived as the difference between V_T in the ROI ($V_{T\ ROI}$) and V_T in the cerebellum ($V_{T\ CER}$), the reference region. The relationship between BP and receptor parameters was given by (Laruelle et al., 1994b)

$$BP = \frac{f_1 B_{max}}{K_D},$$

where B_{max} is the regional concentration of receptor (nmol/L), and K_D is the in vivo affinity of the tracer for the receptor (nmol/L).

The specific to nonspecific equilibrium partition coefficient (V''_3, unitless) was derived as the ratio of BP to $V_{T\ CER}$. The relationship between V''_3 and receptor parameters is given by Laruelle et al. (1994b)

$$V''_3 = \frac{f_2 B_{max}}{K_D},$$

where f_2 is the free fraction of the nonspecific distribution volume in the brain ($f_2 = f_1/V_{T\ CER}$).

Statistical analysis

Unless otherwise stated, values are given as mean ± SD. The effect of RTD on ^{11}C-DASB V_T, BP, and V_3'' was expressed as the percent difference in V_T, BP, and V_3" between the control and RTD conditions (ΔV_T (%), ΔBP (%) and $\Delta V_3''$ (%) = [(RTD value minus control value)/ control value]*100). The significance of differences in V_T, BP, and V_3'' between conditions was analyzed by repeated measures analysis of variance (RM ANOVA) performed in StatView version 5.0.1 (SAS Institute Inc., Cary, NC). Brain region (ROIs and cerebellum) was the factor, and condition (control and RTD) was the repeated measure. Mean values of V_T, BP, and V_3'' were also compared between conditions within each individual brain region by a paired t-test. All statistical tests were two-tailed with a level of significance $\alpha = 0.05$.

In addition, the data were examined for a possible predictive relationship between the changes in plasma Trp/ΣLNAA ratio and ^{11}C-DASB V_T, BP, and V_3'' attributable to RTD across individual subjects. For each subject, the ΔTrp/ΣLNAA (%) value in the RTD condition was subtracted from the ΔTrp/ΣLNAA (%) value in the control condition to give a Δ ΔTrp/ΣLNAA (%) score. This score was considered to correspond to the change in Trp/ΣLNAA attributable to RTD. In addition, a single weighted mean ^{11}C-DASB V_T, BP, and V_3'' was calculated for each subject in each condition by averaging the regional values of V_T, BP, and V_3'' across ROIs. The contribution of each ROI to the mean V_T, BP, and V_3'' values was weighted by its fractional contribution to the total volume of the ROIs when combined. The percent change in the weighted mean values between the control and RTD conditions was then calculated for V_T, BP, and V_3'', giving a single weighted mean ΔV_T (%), ΔBP (%), and $\Delta V_3''$ (%) value across ROIs for each subject. These weighted mean Δ values were considered to be a global measure of the changes in ^{11}C-DASB V_T, BP, and V_3'' attributable to RTD across multiple brain regions. Values across subjects were entered into a simple linear regression analysis, with Δ ΔTrp/ ΣLNAA as independent variable (predictor) and weighted mean ΔV_T, ΔBP, and $\Delta V_3''$ values as dependent variables (outcomes).

Plasma amino acid concentrations before and 6.5 hours after the control (T+) and RTD (T−) mixtures were compared by paired t-test (two-tailed). Any mood change attributable to the amino acid mixture and study protocol was measured as the difference between the Happy-Sad VAMS scores before and 6.5 hours after the amino acid mixture, to give a ΔVAMS score (mm) for each study day. Positive ΔVAMS scores represented a sadder mood, and negative ΔVAMS score represented a happier mood. For each subject, the ΔVAMS score for the RTD day was subtracted from the ΔVAMS score for the control day, to give a Δ ΔVAMS (mm) score. This score was considered to correspond to the mood change attributable to RTD, as the two study days were as similar as possible in all respects other than the composition of the amino acid mixture. ΔVAMS scores associated with the control and RTD conditions were compared across subjects by paired t-test.

RESULTS

Human subjects

All 8 subjects completed both ^{11}C-DASB scans. The protocol and amino acid mixtures were generally well tolerated. Subjects were euthymic on entry to the study (BDI score, 4.1 ± 4.4), and there was no significant effect of RTD on mood (Δ ΔVAMS score = + 6.8 ± 16.9 mm; paired t-test, $P = 0.29$). Most subjects felt the drinks were moderately unpalatable and experienced mild, transient epigastric fullness following their ingestion. Several subjects felt mild transient nausea for approximately 15 minutes following the drinks. One (female) subject vomited on both occasions. On the first day (control), she vomited 2 hours post ingestion, and on the second day (RTD) 2.75 hours post ingestion, despite the amino acid mixture having been reduced to 60 g as a prophylactic measure. Because the vomiting was delayed rather than immediate, and because the subject wished to continue, she was permitted to complete the protocol.

Scan parameters

Across all subjects and both study days, the mean start time for the ^{11}C-DASB scan in the control condition was 7.1 ± 1.9 hours post-amino acid mixture, and in the RTD condition was 6.9 ± 1.8 hours post-amino acid mixture. There was no significant difference in these start times between conditions (paired t-test; $P = 0.52$).

Scan parameters, including the injected dose, specific activity, injected mass of tracer, plasma free fraction, plasma clearance, and nonspecific distribution volume (as measured by $V_{T\ CER}$) did not differ between the control and RTD conditions (Table I).

Distribution volumes and receptor parameters

Figure 1 shows ^{11}C-DASB scans in the control and RTD conditions in the same representative subject. In both conditions, activity concentrated in regions with high SERT densities (MID, THA, CAU, and PUT). Intermediate levels of activity were seen in the AMY, hippocampus, cingulate, and parahippocampal gyrus. Lower levels were observed in the neocortex and CER.

Values of regional ^{11}C-DASB V_T, BP, and V_3'' in the control and RTD conditions are shown in Table II. Figure 2 shows mean ΔV_T, ΔBP, and $\Delta V_3''$ between the control and RTD conditions across ROIs.

RTD significantly altered ^{11}C-DASB V_T (RM ANOVA; ^{11}C-DASB V_T as dependent variable; control

TABLE I. Scan parameters

Condition	ID (mCi)	SA (mCi/μmol)	Mass (μg)	f_1	Cl (L/hr)	$V_{T\ CER}$
T+	14.2 ± 3.9	1272 ± 826	4.1 ± 2.2	0.094 ± 0.016	154 ± 82	10.3 ± 1.6
T−	15.5 ± 1.4	1711 ± 878	3.3 ± 1.9	0.088 ± 0.013	159 ± 19	9.7 ± 1.6
*P**	0.33	0.18	0.39	0.41	0.88	0.17

ID, injected dose of radioactivity; SA, decay-corrected specific activity at time of tracer injection; f_1, plasma free fraction of tracer; Cl, clearance; $V_{T\ CER}$, total distribution volume in region of reference (cerebellum).
*Significance of difference between means assessed by paired t-test (2-tailed).

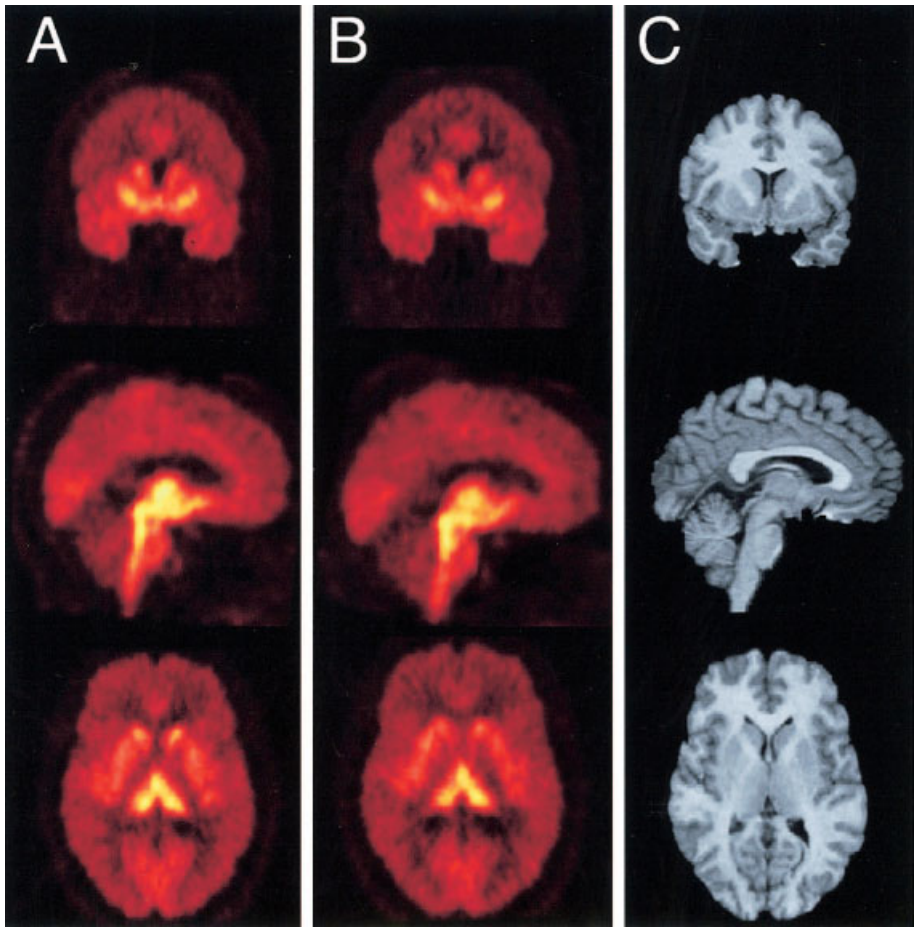

Fig. 1. Coronal (top), sagittal (middle) and transverse (bottom) coregistered PET images acquired from 40 to 90 min following injection of ^{11}C-DASB in a representative 34 year old healthy female volunteer, under control (**A**) and reduced endogenous 5-HT (**B**) conditions. In both scans, ^{11}C-DASB activity concentration is evident in midbrain, thalamus, caudate and putamen. Also illustrated are the low level of activity in the cerebellum, and the small difference in uptake between the cerebellum and neocortical regions. The PET images in panels A and B are normalized to their respective injected doses of activity and displayed with the same intensity range. (**C**) Coregistered MRI images of same subject.

versus RTD condition, $P = 0.0009$; condition*region interaction, $P = 0.94$). The regional average ΔV_T was $-6.1 \pm 1.1\%$ (n = 8, range -4.6% to -7.9%; Fig. 2A). Regional comparisons of mean ΔV_T between conditions (paired t-test) failed to reach significance in any individual region.

RTD significantly altered ^{11}C-DASB BP (RM ANOVA; ^{11}C-DASB BP as dependent variable; control versus RTD condition, $P = 0.01$; condition*region interaction, $P = 0.89$). The regional average ΔBP was $-4.5 \pm 3.3\%$ (n = 7, range from -8.5% to $+2.1\%$; Fig. 2B). Regional comparisons of mean ΔBP between conditions (paired t-test) failed to reach significance in any individual region.

There was no significant effect of RTD on ^{11}C-DASB V''_3 (RM ANOVA; ^{11}C-DASB V''_3 as dependent variable; control versus RTD condition, $P = 0.32$; condition*region interaction, $P = 0.99$). The regional average $\Delta V''_3$ was $+1.0 \pm 3.2\%$ (n = 7, range from -3.2% to $+7.1\%$; Fig. 2C). Regional comparisons of mean $\Delta V''_3$ between conditions (paired t-test) failed to reach significance in any individual region.

TABLE II. Regional total distribution volumes and receptor binding parameters for control and RTD conditions

	CONTROL (T+)			RTD (T−)		
Region	V_T (ml g^{-1})	BP (ml g^{-1})	V''_3	V_T (ml g^{-1})	BP (ml g^{-1})	V''_3
CER	10.3 ± 1.6	N/A	N/A	9.7 ± 1.6	N/A	N/A
AMY	22.7 ± 7.0	12.2 ± 5.6	1.1 ± 0.4	20.5 ± 5.9	10.7 ± 4.4	1.1 ± 0.3
ENT	16.3 ± 5.6	5.8 ± 4.2	0.5 ± 0.3	15.2 ± 4.7	5.4 ± 3.2	0.5 ± 0.2
MID	34.4 ± 13.5	23.9 ± 12.0	2.2 ± 0.8	31.9 ± 9.6	22.1 ± 8.1	2.2 ± 0.5
THA	20.5 ± 4.3	10.1 ± 2.8	1.0 ± 0.2	19.0 ± 4.3	9.2 ± 2.7	0.9 ± 0.1
CAU	17.0 ± 3.5	6.5 ± 2.4	0.6 ± 0.2	15.7 ± 3.8	5.9 ± 2.6	0.6 ± 0.2
PUT	21.5 ± 4.9	11.0 ± 3.7	1.0 ± 0.3	19.8 ± 4.9	10.0 ± 3.6	1.0 ± 0.3
VST	23.5 ± 6.0	13.0 ± 4.5	1.2 ± 0.2	21.9 ± 5.2	12.1 ± 3.7	1.2 ± 0.2

V_T, total distribution volume; BP, binding potential; V''_3, specific-to-nonspecific partition coefficient; RTD, rapid tryptophan depletion; CER, cerebellum; AMY, amygdala; ENT, entorhinal cortex; MID, midbrain; THA, thalamus; CAU, caudate nucleus; PUT, putamen; VST, ventral striatum; N/A, the region of reference (CER) is assumed to have neglible specific binding.

As V_T and BP are proportional to f_1, and V''_3 is not, this suggests that the mean reductions in V_T (−6.1%) and BP (−4.5%) across regions between the control and RTD conditions, in the absence of a change in V''_3, may be attributable to the small concomitant mean reduction in f_1 between conditions, rather than to changes in SERT binding. Although the measured reduction in f_1 between control and RTD conditions was small and was not statistically significant (0.094 vs. 0.088; see Table I), it was of a similar magnitude (−6.3%) to the reduction in V_T. Moreover, assuming f_2 remained constant, a reduction in f_1 should result in a reduction in $V_{T\,CER}$ (i.e., V_2), as $V_2 = f_1/f_2$. In fact, $V_{T\ CER}$ was reduced by a similar mean of −5.9%, although this also failed to reach statistical significance by paired t-test. Furthermore, when values of V_T and BP from each study were corrected for the measured f_1 from that study, there was no significant effect of RTD on ^{11}C-DASB V_T/f_1 (RM ANOVA; ^{11}C-DASB V_T/f_1 as dependent variable; control vs. RTD condition, $P = 0.29$; condition*region interaction, $P = 1.0$; regional average $\Delta V_T/f_1 = +2.8 \pm 1.5\%$, range from +0.8% to +4.7%; data not shown) or BP/f_1 (RM ANOVA; ^{11}C-DASB BP/f_1 as dependent variable; control vs. RTD condition, $P = 0.24$; condition*region interaction, $P = 1.0$; regional average $\Delta BP/f_1 = +4.7 \pm 3.9\%$, range −0.3% to +12.5%; data not shown).

Plasma amino acid assays

Plasma concentrations of total Trp and free Trp, and the Trp/ΣLNAA ratio are shown in Table III. Following the control (T+) drink, plasma total and free Trp concentrations increased by +13.6 ± 64.5% and +76.4 ± 102.9%, respectively. The increase in total Trp was not significant (paired t-test, $P = 0.78$) and the increase in free Trp reached trend significance ($P = 0.06$). Following the RTD (T−) drink, total and free plasma Trp concentrations decreased significantly, by −73.5 ± 22.7% ($P = 0.0002$) and −61.5 ± 45.9% ($P = 0.007$), respectively.

Following the T− drink, the Trp/ΣLNAA ratio decreased by ≥90% in six of the eight subjects. For the other two subjects, one (the female who vomited several hours after ingesting the drink) had a more modest reduction (−68.2%), and the other (24-year-old male) had an unexplained modest reduction of −48.2%. Across all eight subjects, there was a large mean decrease in the Trp/ΣLNAA ratio (−85.1 ± 17.7%; $P = 0.00001$). Following the control (T+) drink, a significant (though less pronounced) decrease in the Trp/ΣLNAA ratio was also observed (−49.1 ± 14.0%; $P = 0.00003$). However, the reduction in the Trp/ΣLNAA ratio was significantly greater following the T-drink than the T+ drink ($P = 0.006$). The changes in total Trp, free Trp, and the Trp/ΣLNAA ratio following the T+ and T-drinks are shown in Figure 3.

Relationship between changes in the plasma Trp/ΣLNAA ratio and brain receptor binding parameters

Across individuals, there was no significant predictive relationship between the Δ ΔTrp/ΣLNAA ratio (i.e., the magnitude of the change in Trp/ΣLNAA attributable to RTD) and mean ΔV_T ($r^2 = 0.116$; $P = 0.41$), ΔBP ($r^2 = 0.007$; $P = 0.84$), and $\Delta V''_3$ ($r^2 = 0.082$; $P = 0.49$) values (i.e., the magnitude of the change in ^{11}C-DASB V_T, BP, and V''_3 attributable to RTD across multiple brain regions).

DISCUSSION

This study investigated the effects of reduced synaptic 5-HT on the binding of the SERT-selective PET radiotracer ^{11}C-DASB in humans, using RTD to reduce synaptic 5-HT. ^{11}C-DASB binding under the condition of reduced 5-HT was compared to binding in a control condition in the same healthy subjects. Animal data suggest that increased 5-HT leads to translocation of SERT to the plasma membrane, while reduced 5-HT leads to the opposite result. It was, therefore, hypothesized that reduced 5-HT in this study would lead to decreased SERT density in plasma membrane and possibly decreased ^{11}C-DASB in vivo specific binding.

The protocol was well tolerated and was completed by all 8 subjects. There were no systematic differences between conditions or scan parameters. RTD was associated with a small but significant mean reduction in ^{11}C-DASB V_T (−6.1%) and BP (−4.5%) values across

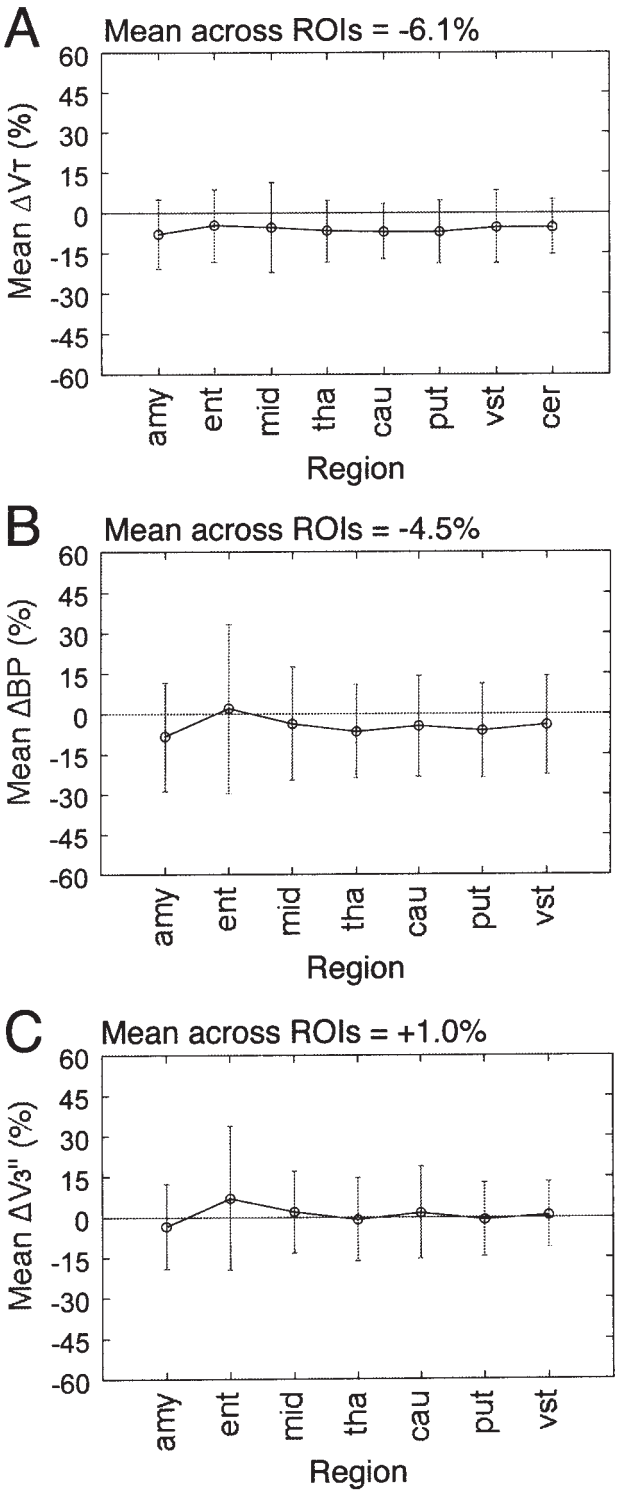

Fig. 2. Interaction line charts showing the mean regional percent changes in ^{11}C-DASB V_T (**A**), BP (**B**) and V_3'' (**C**) between the control and RTD conditions across regions of interest (ROIs). These mean Δ (%) scores represent the change in V_T, BP and V_3'' attributable to rapid tryptophan depletion (RTD). Error bars are 95% confidence intervals. RTD significantly altered ^{11}C-DASB V_T (RM ANOVA; control versus RTD condition, $P = 0.0009$; regional average $\Delta V_T = -6.1 \pm 1.1\%$) and BP ($P = 0.01$; average $\Delta BP = -4.5 \pm 3.3\%$). There was no significant effect of RTD on V_3'' ($P = 0.32$; average $\Delta V_3'' = +1.0 \pm 3.2\%$). Regional comparisons of mean ΔV_T, ΔBP and $\Delta V_3''$ between conditions (paired t-test) failed to reach significance in any individual region.

brain regions (Fig. 2A,B). However, there was no change in ^{11}C-DASB specific binding as measured by V_3'' under conditions of reduced 5-HT (Fig. 2C), suggesting that the reduction in V_T and BP values across brain regions was not due to altered receptor availability under the condition of reduced endogenous 5-HT. Rather, it is likely to be explained by the concomitant (statistically non-significant) difference in f_1 of similar magnitude between the two conditions, and when the values of V_T and BP from each study were corrected for the measured f_1 from that study, the effect of RTD on V_T or BP became statistically non-significant. Moreover, although BP is susceptible to alterations in f_1, V_3'' is not. Thus, the lack of change in ^{11}C-DASB V_3'' under the condition of reduced 5-HT is the clearest indication that ^{11}C-DASB specific binding is not susceptible to alterations in endogenous neurotransmitter at the level of difference between conditions observed in this study. The mechanism by which RTD may cause a small reduction in ^{11}C-DASB f_1 is unexplained.

One possible explanation for the lack of change in ^{11}C-DASB specific binding between conditions would be that RTD was ineffective in reducing synaptic 5-HT in this study. However, this is unlikely to be a valid explanation. Following the RTD (T−) mixture, the plasma Trp/ΣLNAA ratio was decreased by −85.1% at 6.5 hours. As Trp entry to the CNS is controlled by the plasma Trp/ΣLNAA ratio, rather than the absolute concentration of plasma Trp, it is to be assumed that this resulted in a subsequent significant reduction in brain Trp. This is supported by studies of RTD in healthy humans showing that a nadir in plasma Trp of similar magnitude at 5–6 hours is followed by a comparable nadir in CSF Trp at approximately 8 hours post T− mixture (Carpenter et al., 1998; Klaassen et al., 1999; Williams et al., 1999). Furthermore, animal studies have demonstrated that existing brain 5-HT is rapidly depleted in the subsequent few hours. Thus, ^{11}C-DASB binding measured between approximately 5 and 9.5 hours after ingestion of the T− mixture in this study is very likely to have been measured under conditions of significantly reduced endogenous 5-HT.

However, it is evident from the results of the plasma amino acid assays that the control (T+) condition was not completely neutral and that the subjects may have been somewhat 5-HT depleted in the control condition. Following the T+ mixture, the plasma Trp/ΣLNAA ratio was reduced by −49.1%, despite modest increases in total and free plasma Trp (Fig. 3). Thus, a T+ control mixture with 2.2 g Trp, as used in this study, does not appear to contain enough Trp in comparison to the other LNAAs to be a neutral control. This is in agreement with studies also showing reduced plasma Trp/ΣLNAA ratio following control mixtures containing 2.3 g (Wolfe et al., 1995) and 3.0 g (Klaassen et al., 2002; Riedel et al., 1999) of Trp. Therefore, when contrasted with the control (T+) condition, the effects of

TABLE III. Plasma amino acids

	Control (T+) condition			RTD (T−) condition			
Assay	Baseline	Post T+	Δ (%)	Baseline	Post T−	Δ (%)	ΔΔ (%)
Total Trp (nmol/mL)	57.2 ± 11.9	60.3 ± 24.2	+13.6 ± 64.5	55.5 ± 7.7	14.0 ± 11.5	−73.5 ± 22.7**	91.8 ± 80.4
Free Trp (nmol/mL)	6.4 ± 2.5	10.3 ± 4.8	+76.4 ± 102.9	6.7 ± 1.4	2.3 ± 2.4	−61.5 ± 45.9*	−146.6 ± 128.7
Trp/ΣLNAA (%)	10.9 ± 1.4	5.4 ± 0.9	−49.1 ± 14.0***	10.5 ± 1.3	1.6 ± 1.9	−85.1 ± 17.7****	−35.9 ± 26.0

RTD, rapid tryptophan depletion; Trp, tryptophan; LNAA, large neutral amino acids; Trp/ΣLNAA, the ratio of Trp concentration to the sum of the concentrations of LNAAs. ΔΔ (%), the difference between the Δ (%) scores in the control and RTD conditions.
*$P \leq 0.01$; **$P \leq 0.001$; ***$P \leq 0.0001$; ****$P \leq 0.00001$.

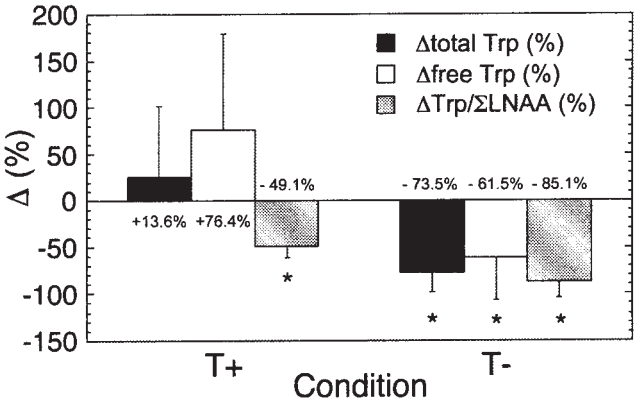

Fig. 3. Mean changes in plasma total tryptophan (Trp), free Trp and the Trp/ΣLNAA ratio 6.5 hr after ingesting the control (T+) and RTD (T−) amino acid mixtures. Error bars represent 1 SD. *Significantly ($P \leq 0.05$) different from baseline measurements. See text and Table III for details.

the RTD (T−) condition on ^{11}C-DASB binding in this study are likely to have under-estimated the effects of reduced endogenous 5-HT.

This raises the possibility that the design of this study might have obscured a potential effect of RTD on ^{11}C-DASB specific binding. However, this is unlikely. First, there was no significant predictive relationship between the magnitude of the change in Trp/ΣLNAA ratio and change in V_T, BP, or V_3'' attributable to RTD across individual subjects. Second, the effects of the Trp content of the control (T+) drink on the subsequent Trp/ΣLNAA ratio have been relatively under-investigated. Indeed, most RTD studies have used a control mixture containing 2.2–2.3 g Trp and have not measured the Trp/ΣLNAA ratio. Thus, the many studies demonstrating significant clinical, cognitive, and physiological changes following RTD (Moore et al., 2000) have used T+ and T− mixtures very similar to those used in this study. It is, therefore, clear that the technique commonly used results in a meaningful reduction in brain 5-HT. However, it is also clear that future RTD studies should attempt to establish a demonstrably neutral control condition. Thus, a Trp content of the control (T+) drink that results in a neutral, rather than modestly depleting, effect on the plasma Trp/ΣLNAA ratio should be established. In addition, a baseline scan without any pharmacological intervention could be added to this type of protocol.

In conclusion, ^{11}C-DASB in vivo specific binding is not sensitive to the decreased concentration of synaptic 5-HT associated with a typically-conducted RTD protocol, and the data reported here do not support the potential of ^{11}C-DASB as an imaging tool to study fluctuations in endogenous 5-HT. However, the results of this study suggest that ^{11}C-DASB may be used to measure differences in SERT availability between populations or conditions without the need to consider concomitant changes in neurotransmitter concentration.

ACKNOWLEDGMENTS

The authors thank Ingrid Gelbard-Stokes, Elizabeth Hackett, John Kim, Kim Ngo, Chaka Peters, Nurat Quadri, Celeste Reinking, Lyudmilla Savenkova, and Zohar Zephrani for their excellent technical assistance. In addition, the invaluable administrative and technical assistance of the late Susan Curry (1977–2004) is respectfully acknowledged. This work was supported in part by a NARSAD young investigator award (P.S.T.).

REFERENCES

Abi-Dargham A, Laruelle M, Seibyl J, Rattner Z, Baldwin RM, Zoghbi SS, Zea-Ponce Y, Bremner JD, Hyde TM, Charney DS, Hoffer PB, Innis RB. 1994. SPECT measurement of benzodiazepine receptors in human brain with [123-I]iomazenil: kinetic and equilibrium paradigms. J Nucl Med 35:228–238.

Abi-Dargham A, Simpson N, Kegeles L, Parsey R, Hwang DR, Anjilvel S, Zea-Ponce Y, Lombardo I, Van Heertum R, Mann JJ, Foged C, Halldin C, Laruelle M. 1999. PET studies of binding competition between endogenous dopamine and the D1 radiotracer [11C]NNC 756. Synapse 32:93–109.

Abi-Dargham A, Martinez D, Mawlawi O, Simpson N, Hwang DR, Slifstein M, Anjilvel S, Pidcock J, Guo NN, Lombardo I, Mann JJ, Van Heertum R, Foged C, Halldin C, Laruelle M. 2000. Measurement of striatal and extrastriatal dopamine D1 receptor binding potential with [11C]NNC 112 in humans: validation and reproducibility. J Cereb Blood Flow Metab 20:225–243.

Abi-Dargham A, Mawlawi O, Lombardo I, Gil R, Martinez D, Huang Y, Hwang DR, Keilp J, Kochan L, Van Heertum R, Gorman JM, Laruelle M. 2002. Prefrontal dopamine D1 receptors and working memory in schizophrenia. J Neurosci 22:3708–3719.

Backstrom I, Bergstrom M, Marcusson J. 1989. High affinity [3H]paroxetine binding to serotonin uptake sites in human brain tissue. Brain Res 486:261–268.

Beck A, Ward C, Mendelson M, Mock J, Erbaugh J. 1961. An inventory for measuring depression. Arch Gen Psychiatry 4:561–571.

Bel N, Artigas F. 1996. Reduction of serotonergic function in rat brain by tryptophan depletion: effects in control and fluvoxamine-treated rats. J Neurochem 67:669–676.

Bender DA. 1985. The transport of amino acids across membranes. In: Bender DA, editor. Amino acid metabolism, 2nd ed. New York: John Wiley & Sons. p 68–70.

Blakely RD, Ramamoorthy S, Schroeter S, Qian Y, Apparsundaram S, Galli A, DeFelice LJ. 1998. Regulated phosphorylation and trafficking of antidepressant-sensitive serotonin transporter proteins. Biol Psychiatry 44:169–178.

Carpenter LL, Anderson GM, Pelton GH, Gudin JA, Kirwin PDS, Price LH, Heninger GR, McDougle CJ. 1998. Tryptophan depletion during continuous CSF sampling in healthy human subjects. Neuropsychopharmacology 19:26–35.

De Haes JI, Bosker FJ, Van Waarde A, Pruim J, Willemsen AT, Vaalburg W, Den Boer JA. 2002. 5-HT(1A) receptor imaging in the human brain: effect of tryptophan depletion and infusion on [(18)F]MPPF binding. Synapse 46:108–115.

Duvernoy H. 1991. The human brain. Surface, three-dimensional sectional anatomy and MRI. New York: Sringer-Verlag Wien.

Folstein MF, Luria R. 1973. Reliability, validity, and clinical application of the Visual Analogue Mood Scale. Psychol Med 3:479–486.

Frankle WG, Slifstein M, Hwang D-R, Huang Y, Talbot PS, Narendran R, Gelbard-Stokes I, Abi-Dargham A, Laruelle M. 2003. Reproducibility of derivation of serotonin transporter parameters with [11C]DASB in healthy humans: comparison of methods. J Nucl Med 44(Suppl):68 p.

Frankle WG, Huang Y, Hwang D-R, Talbot PS, Slifstein M, Van Heertum R, Abi-Dargham A, Laruelle M. 2004. Comparative evaluation of serotonin transporter radioligands [11C]DASB and [11C]McN 5652 in healthy humans. J Nucl Med 45:682–694.

Gandelman MS, Baldwin RM, Zoghbi SS, Zea-Ponce Y, Innis RB. 1994. Evaluation of ultrafiltration for the free-fraction determination of single photon emission computed tomography (SPECT) radiotracers: beta-CIT, IBF, and iomazenil. J Pharm Sci 83:1014–1019.

Gartside SE, Cowen PJ, Sharp T. 1992. Evidence that the large neutral amino acid L-valine decreases electrically-evoked release of 5-HT in rat hippocampus in vivo. Psychopharmacology (Berl) 109: 251–253.

Ginovart N, Wilson AA, Meyer JH, Hussey D, Houle S. 2001. Positron emission tomography quantification of [(11)C]-DASB binding to the human serotonin transporter: modeling strategies. J Cereb Blood Flow Metab 21:1342–1353.

Hariharan M, Naga S, VanNoord T. 1993. Systematic approach to the development of plasma amino acid analysis by high-performance liquid chromatography with ultraviolet detection with precolumn derivatization using phenyl isothiocyanate. J Chromatogr 621:15–22.

Hirani E, Sharp T, Sprakes M, Grasby P, Hume S. 2003. Fenfluramine evokes 5-HT2A receptor-mediated responses but does not displace [11C]MDL 100907: small animal PET and gene expression studies. Synapse 50:251–260.

Houle S, Ginovart N, Hussey D, Meyer J, Wilson A. 2000. Imaging the serotonin transproter with positron emission tomomgraphy: initial human studies with [11C]DAPP and [11C]DASB. Eur J Nucl Med 27:1719–1722.

Huang Y, Hwang DR, Narendran R, Sudo Y, Chatterjee R, Bae SA, Mawlawi O, Kegeles LS, Wilson AA, Kung HF, Laruelle M. 2002. Comparative Evaluation in Nonhuman Primates of Five PET Radiotracers for Imaging the Serotonin Transporters: [11C]McN 5652, [11C]ADAM, [11C]DASB, [11C]DAPA, and [11C]AFM. J Cereb Blood Flow Metab 22:1377–1398.

Hume S, Hirani E, Opacka-Juffry J, Myers R, Townsend C, Pike V, Grasby P. 2001. Effect of 5-HT on binding of [(11)C] WAY 100635 to 5-HT(IA) receptors in rat brain, assessed using in vivo microdialysis and PET after fenfluramine. Synapse 41:150–159.

Kates WR, Abrams MT, Kaufmann WE, Breiter SN, Reiss AL. 1997. Reliability and validity of MRI measurement of the amygdala and hippocampus in children with fragile X syndrome. Psychiat Res 75:31–48.

Killiany RJ, Moss MB, Nicholson T, Jolez F, Sandor T. 1997. An interactive procedure for extracting features of the brain from magnetic resonance images: the lobes. Hum Brain Mapp 5:355–363.

Klaassen T, Riedel WJ, van Someren A, Deutz NE, Honig A, van Praag HM. 1999. Mood effects of 24-hour tryptophan depletion in healthy first-degree relatives of patients with affective disorders. Biol Psychiat 46:489–497.

Klaassen T, Riedel WJ, Deutz NE, Van Praag HM. 2002. Mood congruent memory bias induced by tryptophan depletion. Psychol Med 32:167–172.

Koeppe RA, Holthoff VA, Frey KA, Kilbourn MR, Kuhl DE. 1991. Compartmental analysis of [^{11}C]flumazenil kinetics for the estimation of ligand transport rate and receptor distribution using positron emission tomography. J Cereb Blood Flow Metab 11:735–744.

Laruelle M. 2000. Imaging synaptic neurotransmission with in vivo binding competition techniques: a critical review. J Cereb Blood Flow Metab 20:423–451.

Laruelle M, Huang Y. 2001. Vulnerability of positron emission tomography radiotracers to endogenous competition. New insights. Q J Nucl Med 45:124–138.

Laruelle M, Vanisberg M, Maloteaux J. 1988. Regional and subcellular localization in human brain of [^{3}H]paroxetine binding, a marker of serotonin uptake sites. Biol Psychiat 24:299–309.

Laruelle M, Baldwin RM, Rattner Z, Al-Tikriti MS, Zea-Ponce Y, Zoghbi SS, Charney DS, Price JC, Frost JJ, Hoffer PB, Innis RB. 1994a. SPECT quantification of [123I]iomazenil binding to benzodiazepine receptors in nonhuman primates. I. Kinetic modeling of single bolus experiments. J Cereb Blood Flow Metab 14:439–452.

Laruelle M, van Dyck C, Abi-Dargham A, Zea-Ponce Y, Zoghbi SS, Charney DS, Baldwin RM, Hoffer PB, Kung HF, Innis RB. 1994b. Compartmental modeling of iodine-123-iodobenzofuran binding to dopamine D_2 receptors in healthy subjects. J Nucl Med 35:743–754.

Laruelle M, Slifstein M, Huang Y. 2003. Relationships between radiotracer properties and image quality in molecular imaging of the brain with positron emission tomography. Mol Imag Biol 5:363–375.

Maeda J, Suhara T, Ogawa M, Okauchi T, Kawabe K, Zhang MR, Semba J, Suzuki K. 2001. In vivo binding properties of [carbonyl-11C]WAY-100635: effect of endogenous serotonin. Synapse 40:122–129.

Mawlawi OM, Weiss R, Shinn A, Pidcock J, Slifstien M, Laruelle M. 1999. Performance characteristics of a head immobilization device for PET imaging. J Nucleic Med 40:281P.

Mawlawi O, Martinez D, Slifstein M, Broft A, Chatterjee R, Hwang DR, Huang Y, Simpson N, Ngo K, Van Heertum R, Laruelle M. 2001. Imaging human mesolimbic dopamine transmission with positron emission tomography: I. Accuracy and precision of D2 receptor parameter measurements in ventral striatum. J Cereb Blood Flow Metab 21:1034–1057.

Mintun MA, Raichle ME, Kilbourn MR, Wooten GF, Welch MJ. 1984. A quantitative model for the in vivo assessment of drug binding sites with positron emission tomography. Ann Neurol 15:217–227.

Moore P, Landolt HP, Seifritz E, Clark C, Bhatti T, Kelsoe J, Rapaport M, Gillin JC. 2000. Clinical and physiological consequences of rapid tryptophan depletion. Neuropsychopharmacology 23:601–622.

Pani L, Gessa GL, Carboni S, Portas CM, Rossetti ZL. 1990. Brain dialysis and dopamine: does the extracellular concentration of dopamine reflect synaptic release? Eur J Pharmacol 180:85–90.

Plenge P, Mellerup ET, Laursen H. 1990. Regional distribution of the serotonin transport complex in human brain, identified with 3H-paroxetine, 3H-citalopram and 3H-imipramine. Prog Neuropsychopharmacol Biol Psychiat 14:61–72.

Rabiner EA, Messa C, Sargent PA, Husted-Kjaer K, Montgomery A, Lawrence AD, Bench CJ, Gunn RN, Cowen P, Grasby PM. 2002. A database of [(11)C]WAY-100635 binding to 5-HT(1A) receptors in normal male volunteers: normative data and relationship to methodological, demographic, physiological, and behavioral variables. Neuroimage 15:620–632.

Rice OV, Gatley SJ, Shen J, Huemmer CL, Rogoz R, DeJesus OT, Volkow ND, Gifford AN. 2001. Effects of endogenous neurotransmitters on the in vivo binding of dopamine and 5-HT radiotracers in mice. Neuropsychopharmacology 25:679–689.

Riedel WJ, Klaassen T, Deutz NE, van Someren A, van Praag HM. 1999. Tryptophan depletion in normal volunteers produces selective impairment in memory consolidation. Psychopharmacology (Berl) 141:362–369.

Staley JK, Van Dyck CH, Tan PZ, Al Tikriti M, Ramsby Q, Klump H, Ng C, Garg P, Soufer R, Baldwin RM, Innis RB. 2001. Comparison of [(18)F]altanserin and [(18)F]deuteroaltanserin for PET imaging of serotonin(2A) receptors in baboon brain: pharmacological studies. Nucl Med Biol 28:271–279.

Stancampiano R, Melis F, Sarais L, Cocco S, Cugusi C, Fadda F. 1997. Acute administration of a tryptophan-free amino acid mixture decreases 5-HT release in rat hippocampus in vivo. Am J Physiol 272:R991–R994.

Suehiro M, Scheffel U, Dannals RF, Ravert HT, Ricaurte GA, Wagner H, Jr. 1993. A PET radiotracer for studying serotonin uptake sites: carbon-11-McN-5652Z. J Nucl Med 34:120–127.

Szabo Z, McCann UD, Wilson AA, Scheffel U, Owonikoko T, Mathews WB, Ravert HT, Hilton J, Dannals RF, Ricaurte GA. 2002. Comparison of (+)-(11)C-McN5652 and (11)C-DASB as serotonin transporter radioligands under various experimental conditions. J Nucleic Med 43:678–692.

Talairach J, Tournoux P. 1988. Co-planar stereotactic atlas of the human brain. Three-dimensional proportional system: an approach of cerebral imaging. New York: Theime Medical Publisher. 122 p.

Talbot PS, Laruelle M. 2002. The role of in vivo molecular imaging with PET and SPECT in the elucidation of psychiatric drug action and new drug development. Eur Neuropsychopharmacol 12:503–511.

Williams WA, Shoaf SE, Hommer D, Rawlings R, Linnoila M. 1999. Effects of acute tryptophan depletion on plasma and cerebrospinal

fluid tryptophan and 5-hydroxyindoleacetic acid in normal volunteers. J Neurochem 72:1641–1647.
Wilson AA, Houle S. 1999. Radiosynthesis of carbon-11 labelled N-methyl-2(arylthio)benzylamines: potential radiotracers for the serotonin reuptake receptor. J Label Compd Radiopharm 42:1277.
Wilson AA, Ginovart N, Schmidt M, Meyer JH, Threlkeld PG, Houle S. 2000. Novel radiotracers for imaging the serotonin transporter by positron emission tomography: synthesis, radiosynthesis, and in vitro and ex vivo evaluation of (11)C-labeled 2-(phenylthio)araalkylamines. J Med Chem 43:3103–3110.
Wilson AA, Ginovart N, Hussey D, Meyer J, Houle S. 2002. In vitro and in vivo characterisation of [11C]-DASB: a probe for in vivo measurements of the serotonin transporter by positron emission tomography. Nucleic Med Biol 29:509–515.
Wolfe BE, Metzger ED, Jimerson DC. 1995. Comparison of the effects of amino acid mixture and placebo on plasma tryptophan to large neutral amino acid ratio. Life Sci 56:1395–1400.
Woods RP, Cherry SR, Mazziotta JC. 1992. Rapid automated algorithm for aligning and reslicing PET images. J Comp Assist Tomogr 16:620–633.
Zahniser NR, Doolen S. 2001. Chronic and acute regulation of Na+/Cl− -dependent neurotransmitter transporters: drugs, substrates, presynaptic receptors, and signaling systems. Pharmacol Ther 92: 21–55.
Zimmer L, Mauger G, Le Bars D, Bonmarchand G, Luxen A, Pujol JF. 2002. Effect of endogenous serotonin on the binding of the 5-hT1A PET ligand ^{18}F-MPPF in the rat hippocampus: kinetic beta measurements combined with microdialysis. J Neurochem 80:278–286.
Zimmer L, Rbah L, Giacomelli F, Le Bars D, Renaud B. 2003. A reduced extracellular serotonin level increases the 5-HT1A PET ligand ^{18}F-MPPF binding in the rat hippocampus. J Nucl Med 44:1495–1501.