
*eMethods*. Image Acquisition and Analysis

This supplementary material has been provided by the authors to give readers additional information about their work.
eMethods. Image Acquisition and Analysis

Radiochemistry and Input Function Measurement

Synthesis of $^{[11]C}$WAY-100635 and $^{[11]C}$DASB and assessment of arterial input function, metabolites, and plasma free fraction ($f_P$) measurements were as previously described.$^{29,30}$ Radioligand metabolite data were collected for the first 60 minutes of scan time.

Image Acquisition

PET imaging was performed with a ECAT HR+ (Siemens/CTI, Knoxville, TN, USA). Briefly, a venous catheter was placed for radiotracer injection and an arterial catheter to obtain samples for the input function. A polyurethane head holder system (Soule Medical, Tampa, FL, USA) was molded around the subject's head for immobilization purposes. A 10-minute transmission scan was obtained prior to each radiotracer injection. At the end of the transmission scan, $^{[11]C}$DASB was administered intravenously as a bolus over 30 seconds. Emission data were collected in 3D mode for 100 minutes with 19 frames of increasing duration.$^{31}$ Similarly, emission data for $^{[11]C}$WAY-100635 were collected for 110 minutes as 20 frames of increasing duration after obtaining a transmission scan. Additional details of the PET protocols for $^{[11]C}$DASB and $^{[11]C}$WAY-100635 are described elsewhere.$^{31}$

Image Analysis

Image analysis was performed in MATLAB 2006b (The Mathworks, Natick, MA) using Functional Magnetic Resonance Imaging of the Brain’s Linear Image Registration Tool (FLIRT) v5.2.,$^{32}$ Brain Extraction Tool v1.2,$^{33}$ Statistical Parametric Mapping (SPM5) normalization,$^{34}$ and segmentation routines.$^{35}$ De-noising filter techniques were applied to all PET images starting at frame five to correct for motion. Frames were aligned using rigid body FLIRT to the eighth frame. The mean of motion-corrected frames eight through eighteen was registered to
MRI using FLIRT. T1-weighted magnetic resonance images (MRI) for PET image co-
registration and identification of regions of interest (ROIs) were acquired using a 1.5 T Signa
Advantage or a 3 T Signa HDx system (General Electric, Milwaukee, WI) as previously
described. An automated algorithm was used to obtain ROIs in this study using nonlinear
registration techniques to warp 18 manually outlined MRIs to the target image. At each voxel,
the probability of being within an ROI was calculated as the number of transformed template
ROIs assigned to that voxel divided by 18. Non-midline ROIs were averaged across
hemispheres. In previous work, this approach yielded very high correlations of binding as
compared to those obtained with manually-drawn ROIs (r²>0.98;).

Derivation of Regional Outcome Measures for Midbrain 5-HTT Binding Potential

Since no brain region appears devoid of specific binding with [11C]DASB, our outcome
measure does not rely on a reference region: V_T/f_P (where V_T = volume of distribution in the
region of interest) [for details see Miller et al9]. [11C]DASB regional V_T values were derived using
likelihood estimation in the graphical approach, to reduce its inherent noise-dependent bias.38,39
Brain activity was corrected for the plasma activity contribution, assuming a 5% blood volume
contribution to the region of interest (midbrain). In addition, we calculated other 5-HTT binding
outcome measures: BP_F, BP_P and BP_ND which were examined in sensitivity analyses.

Derivation of Regional Outcome Measures for 5-HT1A ROI Binding Potential

Although the optimal outcome measure in PET neuroreceptor studies is the number of
receptors available (B_avai), current technology only permits measurement of BP_F=B_avai/K_D.
Fortunately, there is no evidence for alterations in 5-HT1A K_D in depression.41 Kinetic analysis
using an arterial input function and a two-tissue compartment (2T) model yields regional
volumes of (V) of distribution for [11C]WAY-100635. V_ND and V_S are the distribution volumes of
the nondisplaceable and specific compartments, respectively.40 V_T is the total regional
equilibrium distribution volume, equal to the sum of V_ND and V_S. Time activity curves are fit with
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a 2T model, with the $K_1/K_2$ ratio fixed to that of the cerebellar white matter (CWM), a reference region with virtually no 5-HT$_{1A}$ binding. A 1T model was used for CWM. $^{29}$ $BP_F$ was calculated as $(V_T(ROI) - V_T(CWM))/f_P$. Plasma total activity was calculated assuming a 5% blood volume in the ROI and subtracted from the regional activity. Kinetic parameters were derived by nonlinear regression using MATLAB. ROI contours were processed using the segmented MRI to confine analyses to gray matter voxels. The ROIs included: raphe nucleus, amygdala, hippocampus; parahippocampal gyrus, anterior cingulate, medial and dorsolateral PFC, insular, parietal, temporal, orbital and occipital cortex. A fixed volume elliptical ROI (2cm$^3$) was placed on the RN in dorsal midbrain on a mean PET image, as previously described.$^{42}$ A cylindrical ROI was drawn in the CWM.$^{29}$
References


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