Radiochemistry

The purified ^{11}C-(+)-PHNO product was formulated in 1 mL of USP absolute ethanol and 10 mL of USP sterile saline, and 0.04 mL of 4.2% sodium bicarbonate. Filtration of the ethanolic saline solution through a 0.22 μm Millipore membrane filter produced sterile, apyrogenic ^{11}C-(+)-PHNO solution ready for intravenous administration.

Arterial Input Function Measurement

Whole blood and plasma. Both continuous and sequential discrete arterial blood samples were taken as previously described (*1*) with modifications: sample volumes ranged from 2 to 10 mL; the function used to fit the whole-blood-over-plasma ratio was a sum of two exponentials.

Determination of ligand metabolism in plasma. In order to measure the ligand metabolism in plasma, six plasma samples (3, 7, 15, 30, 60 and 90 min postinjection; 2 to 10 mL) were mixed with urea at a final concentration of 8 M and 16 mg of citric acid, and filtered through a Millipore syringe filter (0.45 μm). The filtrate was then analyzed by reverse-phase HPLC using a column-switching system (*2*). Up to 5 mL of filtrate was loaded on the HPLC system (Shimadzu, Kyoto, Japan), and a mobile phase of 1% acetonitrile water was eluted through the self-packed capture column with solid phase extraction C18 sorbent (Strata-X, Phenomenex, Torrance, CA, USA) at a flow rate 2 mL/min. Then the content of the capture column was back-flushed onto a Phenomenex Luna C18 analytical column (250x4.6mm, 5 μm) (Phenomenex, Torrance, CA, USA), with a mobile phase consisting of 77:23 0.1M ammonium formate pH6.4: acetonitrile at a flow rate of 1.4 mL/min for the analytical column. The output of the HPLC column was connected to a fraction collector (CF-1 Fraction Collector, Spectrum Chromatography, Houston, TX, USA). Fractions were collected every two minutes and counted in a cross-calibrated well counter.

The ratio of the radioactivity concentrations in filtrate and plasma was obtained and fitted to an exponential rise to plateau curve ($f_{\mathrm{F}}(t) = a - be^{-\alpha t}$). The unchanged fraction in the filtrate from HPLC was fitted to a bounded sum of exponentials ($f_{\mathrm{H}}(t) = \min\left(1, A_1 e^{-\alpha_1 t} + A_2 e^{-\alpha_2 t}\right)$). The unchanged fraction in plasma was then computed as the product of the functions f_{F} and f_{H}. Finally, the arterial input function was computed as the product of the plasma radioactivity concentration and the unchanged fraction.

Plasma free fraction. The plasma free fraction (f_{P}) of ^{11}C-(+)-PHNO was measured by ultrafiltration as previously described (*1*).

PET Data Acquisition, Image Reconstruction and Motion Correction

During the PET scans, subjects wore a swim cap to which a rigid optical tracking tool was attached to record head motion with an infrared detector (Vicra, NDI Systems, Waterloo, Ontario, Canada). A 6-min transmission scan was acquired after starting recording head motion with the infrared detector and just before ^{11}C-(+)-PHNO injection, for both test and retest scans. List mode data were acquired for 120 minutes following ^{11}C-(+)-PHNO injection. Dynamic list mode data were reconstructed with all corrections (attenuation, normalization, scatter, randoms, deadtime and motion) using the MOLAR algorithm (*3*) with the following frame timing: 6 x 30 sec; 3 x 1 min; 2 x 2 min; 22 x 5 min. A second step of motion correction was also performed, consisting of image smoothing (Gaussian filter with a full width at half maximum (FWHM) of 3 pixels) followed by coregistration to an early summed image (0-10 min post-injection) using a 6-parameter mutual information algorithm (FLIRT, FSL 3.2, Analysis Group, FMRIB, Oxford, UK). The registration of early reference and each frame's image data was weighted using a head mask based on the transmission image and Vicra motion correction data, i.e. the head mask was resliced according to the Vicra motion data, and a weight image was computed for each frame (and for the 0-10 reference period) as the average of all corresponding resliced masks.

Magnetic Resonance Imaging

MR imaging was performed on a 3T Trio (Siemens Medical Systems, Erlangen, Germany) with a circularly-polarized head coil. MR acquisition was a Sag 3D magnetization-prepared rapid gradient-echo (MPRAGE) sequence with 3.34 ms echo time, 2,500 ms repetition time, 1,100 ms inversion time, 7 degree flip angle, and 180 Hz/pixel bandwidth. The image dimensions were 256 x 256 x 176 and pixel size was 0.98 x 0.98 x 1.0 mm.

Regional TAC Computation

Gray matter regions-of-interest (ROIs) were taken from the Anatomical Automatic Labeling (AAL) template (*4*) delineated on a MR template (*5*). Six ROIs were selected: cerebellum (84 cm^3 in template space), caudate (16 cm^3), putamen (17 cm^3), pallidum (4.6 cm^3), amygdala (3.7 cm^3), and thalamus (17 cm^3). Extra ROIs corresponding to the hypothalamus (1.4 cm^3) and ventral striatum (2.2 cm^3) were also drawn on the template MRI. The ventral striatum was drawn similarly to a previous study (*6*). Finally, a SN template ROI (1.0 cm^3) was also created: individual SN ROIs were drawn on each PET scan on SRTM2 binding potential (BP_{ND}) images. ROIs were drawn as a series of ellipsoids on 6-7 coronal slices with a target volume of 1 cm^3 (bilaterally); then a template ROI was created from these individual ROIs by transferring them to template space, creating a probability map by averaging these transferred ROIs, and finally selecting voxels with the highest probability, with a cut-off determined to achieve the desired 1-mL volume.

To apply the ROIs to the PET data, two transformations were estimated. First, a nonlinear transformation grid was estimated between the template MR image and each subject's MR image, using the Bioimagesuite software (version 2.5; http://www.bioimagesuite.com). This nonlinear coregistration algorithm is based on the method proposed by (*7*) with modifications (*8*). Then, a summed image (0-10 min postinjection) was created from the motion-corrected PET dynamic

images and registered to the subject's MR image, using a 6-parameter rigid registration estimated with a mutual information algorithm (FLIRT, FSL 3.2, Analysis Group, FMRIB, Oxford, UK).

A similar approach to delineate ROIs (using the AAL template and coregistrations between the template and subject MRIs, and between the subjects' PET and MR images) was used previously in test-retest studies (*9-11*), with different software.

Parameter Estimation

Parameters were estimated using weighted least squares, with weights based on the noise-equivalent counts in each frame using custom software for IDL 8.0 (ITT Visual Information Solutions, Boulder, CO, USA), as previously described (*1*).

Effect of Scan Duration

The scan duration in this study was 120 min. When only 90-min of data were analyzed, V_T and BP_{ND} estimates were slightly lower for all methods, especially in high BP_{ND} ROIs: e.g., the regression line between 120-min and 90-min V_T estimates (MA1) was $y = 0.91x + 0.07$, with r^2=0.962. With only 90-min of data, the agreement between MA1 and SRTM or SRTM2 BP_{ND} estimates was improved: e.g., the regression equation between MA1 and regional averages of SRTM BP_{ND} images was $y = 1.03x - 0.004$, with r^2=0.947 (versus $y = 0.89x + 0.22$, r^2=0.952, with 120-min data). Finally, the difference in $\sigma(\Delta BP_{ND})$ between 90-min and 120-min scans was less than 1 percentage point in caudate, putamen, ventral striatum and thalamus, but $\sigma(\Delta BP_{ND})$ was 3, 5 and 8 percentage points higher in the pallidum, amygdala and SN, respectively, with only 90-min data. The only ROI with lower $\sigma(\Delta BP_{ND})$ (by 4 percentage points) with 90-min data was the hypothalamus. Therefore on the HRRT scanner, 120-min scans are preferable, but only for applications targeting D3-receptors.

Comparison of various volumes of distribution and binding potentials

Normalizing V_T by f_P did not change TRV: both $m(\Delta V_T)$ and $m(\Delta V_T/f_P)$ ranged between -9% and +2%, and $\sigma(\Delta V_T)$ ranged from 13% to 25% while $\sigma(\Delta V_T/f_P)$ ranged from 13% to 24% (Table S1). Therefore, based on this study, V_T and V_T/f_P can be used interchangeably, but measuring f_P is recommended for studies in which V_T will be compared across different populations or during pharmacological challenges where f_P might be affected.

The binding potentials, BP_{ND}, BP_P and BP_F, computed from MA1 V_T estimates are listed in Table S2. The binding potential with the lowest variability was BP_{ND}, with $\sigma(\Delta BP_{ND})$ ranging from 10% to 29%, and $m(|\Delta BP_{ND}|)$ ranging from 6% to 25%. The variability of BP_P and BP_F was ~22% and ~13% higher, respectively, than that of BP_{ND} (based on $\sigma(\Delta p)$). This lower variability of BP_{ND} compared to BP_P and BP_F was also seen for ^{11}C-raclopride (*6*) and ^{11}C-NPA (*12*).

Table S1: Volumes of distributions estimated with MA1 fits of regional TACs.

	V_T			V_T/f_P		
	Average*	ΔV_T†	ICC‡	Average*	$\Delta V_T/f_P$†	ICC‡
Cerebellum	4.7±0.7(14%)	2%±18%(12%)	0.28[-0.44;0.79]	10.7±1.5(14%)	2%±18%(12%)	0.06[-0.60;0.69]
Caudate	13.2±1.8(14%)	0%±15%(11%)	0.81[0.35;0.96]	30.1±3.6(12%)	0%±15%(11%)	0.76[0.23;0.94]
Putamen	15.8±1.9(12%)	-1%±13%(9%)	0.72[0.16;0.94]	35.9±4.2(12%)	-1%±13%(9%)	0.59[-0.08;0.90]
Pallidum	20.1±3.2(16%)	-2%±14%(9%)	0.88[0.54;0.97]	45.9±7.2(16%)	-2%±14%(9%)	0.84[0.43;0.96]
V. Striatum	22.2±4.9(22%)	0±21%(15%)	0.64[0.01;0.92]	50.6±10.8(21%)	1±19%(15%)	0.60[-0.06;0.90]
Amygdala	5.9±0.8(14%)	-1±15%(10%)	0.51[-0.19;0.88]	13.5±1.6(12%)	0±13%10%)	0.39[-0.33;0.84]
Sub. Nigra	13.4±2.6(19%)	-9%±25%(21%)	0.85[0.45;0.97]	30.5±5.8(19%)	-9%±24%(20%)	0.86[0.50;0.97]
Thalamus	6.4±1.2(18%)	2%±17%(13%)	0.55[-0.13;0.89]	14.6±2.5(17%)	2%±17%(13%)	0.51[-0.19;0.88]
Hypothalamus	12.4±2.7(22%)	-3%±14%(11%)	0.80[0.32;0.95]	28.2±5.5(19%)	-3%±15%(13%)	0.71[0.13;0.93]

*n=5 healthy controls; data are presented as mean±sd (relative sd) across subjects.
†n=8 subjects; data are presented as $m(\Delta p)\pm\sigma(\Delta p)$ ($m(|\Delta p|)$), where p is V_T or V_T/f_P.
‡n=8 subjects; ICC is presented as estimate [lower bound; upper bound] of 95% confidence interval

Table S2: Binding potential estimates for MA1 fits of regional TACs.

	BP_{ND}			BP_P			BP_F		
	Average*	ΔBP_{ND}†	ICC‡	Average*	ΔBP_P†	ICC‡	Average*	ΔBP_F†	ICC‡
Caudate	1.8±0.2(9%)	-2%±10%(9%)	0.92[0.69;0.98]	8.5±1.2(14%)	0%±14%(11%)	0.89[0.59;0.98]	19.4±2.3(12%)	0%±12%(10%)	0.91[0.64;0.98]
Putamen	2.4±0.2(8%)	-4%±11%(9%)	0.57[-0.11;0.89]	11.1±1.3(12%)	-2%±11%(9%)	0.80[0.34;0.96]	25.2±2.8(11%)	-1%±10%(8%)	0.76[0.25;0.95]
Pallidum	3.3±0.6(17%)	-6%±11%(6%)	0.87[0.51;0.97]	15.4±2.9(18%)	-4%±14%(8%)	0.91[0.65;0.98]	35.2±6.4(18%)	-3%±15%(10%)	0.88[0.56;0.97]
V. Striatum	3.7±0.6(15%)	-2±12%(10%)	0.82[0.38;0.96]	17.5±4.3(25%)	0%±22%(17%)	0.69[0.09;0.93]	39.9±9.5(24%)	1%±20%(16%)	0.68[0.07;0.92]
Amygdala	0.26±0.07(26%)	-13±29%(25%)	0.29[-0.42;0.80]	1.2±0.3(28%)	-11%±21%(17%)	0.57[-0.11;0.89]	2.8±0.6(23%)	-10%±17%(14%)	0.63[-0.02;0.91]
Sub. Nigra	1.8±0.4(19%)	-16%±17%(19%)	0.92[0.68;0.98]	8.7±2.2(25%)	-14%±28%(24%)	0.86[0.51;0.97]	19.7±4.8(24%)	-14%±28%(23%)	0.88[0.55;0.97]
Thalamus	0.36±0.10(28%)	2%±19%(14%)	0.73[0.17;0.94]	1.7±0.6(36%)	4%±21%(16%)	0.78[0.29;0.95]	3.9±1.3(34%)	-5%±17%(14%)	0.82[0.39;0.96]
Hypothalamus	1.7±0.8(48%)	-7%±27%(21%)	0.55[-0.14;0.89]	7.7±2.9(38%)	-5%±21%(18%)	0.80[0.34;0.96]	17.5±6.3(36%)	-5%±22%(20%)	0.76[0.23;0.94]

*n=5 healthy controls; data are presented as mean±sd (relative sd) across subjects.

†n=8 subjects; data are presented as $m(\Delta BP_X) \pm \sigma(\Delta BP_X)$ ($m(|\Delta BP_X|)$), where X stands for ND, P, or F.

‡n=8 subjects; ICC is presented as estimate [lower bound; upper bound] of 95% confidence interval

Variability of SRTM2 and SRTM2 Parametric Images

Table S3: Variability of SRTM and SRTM2 parametric images.

	$\sigma(\Delta R_1)^*$		$\sigma(\Delta BP_{ND})^*$	
	SRTM	SRTM2	SRTM	SRTM2
Caudate	25%	14%	34%	22%
Putamen	27%	16%	40%	28%
Pallidum	23%	15%	47%	28%
V. Striatum	22%	15%	39%	28%
Amygdala	37%	23%	155%	86%
Sub. Nigra	20%	18%	46%	38%
Thalamus	31%	19%	83%	48%
Hypothalamus	23%	20%	44%	45%

*Parametric images of $\sigma(\Delta R_1)$ and $\sigma(\Delta BP_{ND})$ were computed in template space (n=8 subjects), and the median value in each ROI is reported.

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