Supplementary Information

Duration Study

In order to determine the optimal timing of tracer injection after oral dosing of MPH, we performed a duration study in two male subjects (ages 34 and 47) to assess the time from MPH oral dosing to maximum occupancy of brain NET. We administered 40 mg of single-blind MPH at 75, 150, and 225 minutes before [^{11}C]MRB injection to compare BP_{ND} with the baseline (0 mg MPH) scan. On day 1, the baseline (0 mg MPH) scan was followed by administration of 40 mg of MPH and a second scan 150 minutes later. On a separate day, one week later, we administered 40 mg of MPH and scanned each subject at 75 and 225 minutes later. The injected dose was 19.8±0.7 mCi. The baseline BP_{ND} in the thalamus was 0.43±0.07. After MPH 40 mg it decreased to 0.25±0.02 at 75 minutes (one-sided t test, p=0.04), to 0.29±0.05 at 150 minutes (one-sided t test, p=0.08), and to 0.12±0.09 at 225 minutes (one-sided t test, p=0.03). This pattern was similar in other NET-rich regions. Based on this pattern there was no significant difference in the values between 75, 150, and 225 min. This is consistent with *peak brain levels occur between 1 and 2 hours, which is the same as peak serum concentration and behavioral effects of clinical doses (6)* . Therefore, we chose 75 minutes as the timing for the occupancy study in order to optimize scan day logistics.

Arterial Input Function Measurement

On the first and last study day of the occupancy study (0 and 2.5 mg, and 40 mg), both continuous and sequential discrete arterial blood samples were taken. For the first 7 minutes, blood was drawn continuously with a peristaltic pump (4 ml/min), and radioactivity in whole blood was measured with a cross-calibrated radioactivity monitor (PBS-101, Veenstra Instruments, Joure, Netherlands). During this period, the pump was briefly stopped to take samples at 3 and 5 minutes. Thereafter, discrete blood samples were obtained at 7, 10, 15, 20, 25, 30, 40, 50, 60, 75, 90, 105, and 120 minutes post-injection. Whole blood and plasma were counted in a cross-calibrated well counter (Wizard 1480, Perkin-Elmer, Waltham, MA). The plasma time-activity curve (TAC) during the first seven minutes was estimated from the continuous whole blood TAC. The ratio of whole-blood to plasma concentration was calculated

for each sample drawn between 3 and 30 minutes, fitted to a linear function, and then extrapolated between 0 and 7 minutes.

The fraction of unchanged [^{11}C]MRB in plasma at 10, 20, 40, 60, and 90 minutes after injection was analyzed by using high performance liquid chromatography (HPLC) assay with acetonitrile extracted plasma samples as previously described (Ding, Lin et al 2005) or a column-switching HPLC method developed by Hilton (1). In brief, the supernatant plasma samples mixed with urea at a final concentration of 8M was filtered through 0.45 mm syringe filter (Millex-HA, Millipore Corp., Bedford, MA, USA). Activity in the filtered plasma sample and the filter were counted. Up to 5 mL of the plasma sample was coinjected with the non-labeled MRB onto the column-switching HPLC system with the analytical mobile phase designated 70: 30 0.1M Ammonium formate pH6.5: acetonitrile at 1.6 mL/min flow rate and Luna C18 phenyl hexyl analytical column (250x4.6mm, 5µm, Phenomenex, Torrance, CA, USA). The MRB parent compound retention time is around 12 min after injection. The signal from the detector is collected by Class-VP 7.1 software (Shimadzu, Kyoto, Japan). All the HPLC eluent was also fraction-collected every two minutes by an automated fraction collection device (CF-1 Fraction Collector, Spectrum Chromatography, Houston, TX, USA).

To estimate the plasma free fraction of [^{11}C]MRB, 740 kBq of [^{11}C]MRB was spiked (<0.2 mL saline / mL blood) into a sample of each subject's blood withdrawn prior to [^{11}C]MRB injection. After mixing and centrifugation to precipitate blood cells, plasma proteins were separated using ultrafiltration tubes (Centrifree UF device number 4104, Millipore, Billerica, MA, USA) and centrifugation (1100g for 20 min; IEC Medilite centrifuge, Thermo Fisher Scientific, Waltham, MA, USA). Plasma and ultrafiltrate samples were counted in triplicate and free fraction (f_p) was estimated as the ratio of the mean concentration in ultrafiltrate and plasma.

Image Analysis

Parametric images of [^{11}C]MRB volume of distribution (V_T) and binding potential (BP_{ND}) values were calculated using MA1 and MRTM2. Equation 1 is the MA1 operational equation:

$$C_T(t) = -\frac{V_T}{b}\int_0 C_P(u)du + \frac{1}{b}\int_0 C_T(u)du, \quad t > t^* \qquad \textbf{Eq. 1}$$

Equation 2 is the MRTM2 operational equation:

$$C_T(t) = -\frac{1+BP_{ND}}{b}\int_0 C_{Ref}(u)du + \frac{1}{b}\int_0 C_T(u)du + \frac{b'}{b}(1+BP_{ND})C_{Ref}(t), \quad t > t^* \qquad \textbf{Eq. 2}$$

In Equation 2, *b*' is a parameter linked to the reference region TAC only. Thus, it should ideally have a single value in across all regions (2). For each subject the common *b*' value was estimated using Equation 2 above with 3-parameter fits (i.e. BP_{ND}, *b*, and *b*') and computing the median of *b*' estimates from all brain voxels. Then, Equation 2 with 2-parameter fits was used to compute the final BP_{ND} parametric images. For both MA1 and MRTM2, the dynamic images were pre-smoothed with a Gaussian filter (FWHM = 3 voxels) and then masked to exclude non-brain voxels. The parameter t* was set to 20 min.

MPH Plasma Levels

The correlation between plasma levels of *d-threo*-MPH and MPH dose (mg/kg) is shown in Supplementary Figure 1 below. The correlation between all plasma levels and doses was significant (C_{MPH} = 35.3 × D_{MPH}, r2 = 0.745, n=29). However, within each group of doses (i.e., 2.5, 10 or 40 mg), the correlations were not significant (at 2.5 mg, C_{MPH} = 33.5 × D_{MPH}, r2 = 0.084, n=10; at 10 mg, C_{MPH} = 25.7 × D_{MPH}, r2 = 0.171, n=10; at 40 mg, C_{MPH} = 36.0 × D_{MPH}, r2 = 0.028, n=9). Therefore, the doses of MPH (in mg/kg) were used as covariates to analyze the binding of [^{11}C]MRB.

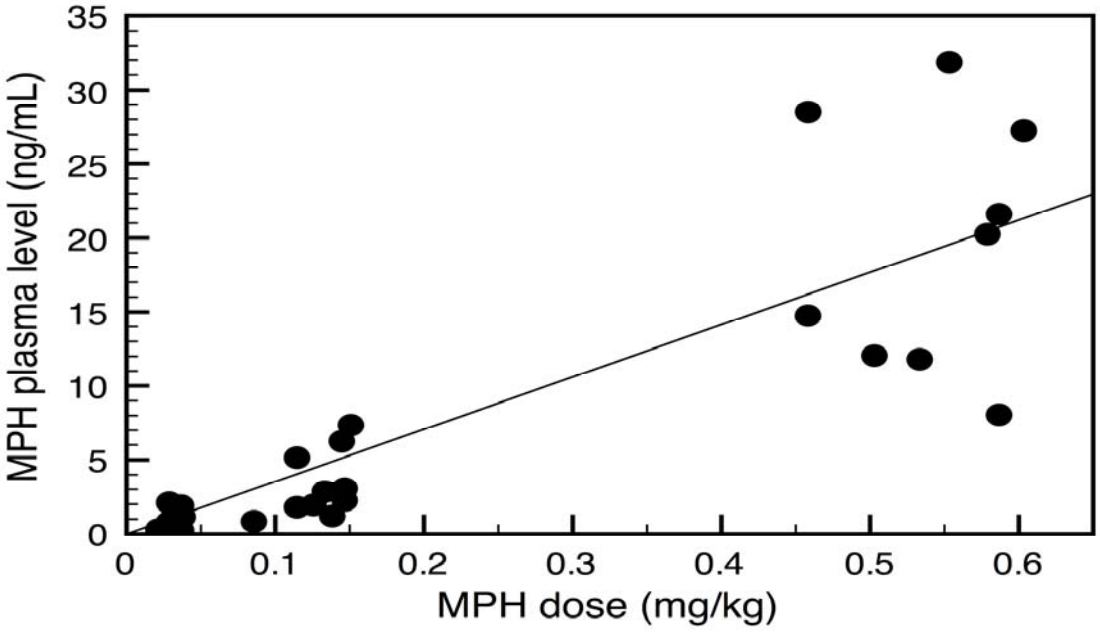

Figure 1. Correlation between the plasma levels of *d-threo*-MPH and the oral dose of MPH ($C_{MPH} = 35.3 \times D_{MPH}$, $r^2 = 0.745$, n=29).

Binding Potential Values by Region and Dose

In Supplementary Table 1 below are the mean values and standard deviations of binding potential (BP) for each region and dose. These are the values reflected in Figure 3.

Table 1. Binding Potential (BP_{ND}) by region and dose

	0 mg	2.5 mg	10 mg	40 mg
Nucleus ruber	0.41±0.18	0.40±0.18	0.27±0.1	0.18±0.1
Locus caeruleus	0.43±0.14	0.35±0.21	0.17±0.1	0.05±0.12
Raphé midbrain	0.63±0.24	0.6±0.25	0.35±0.13	0.12±0.15
Raphé pons	0.56±0.27	0.51±0.30	0.22±0.12	011±0.13
Hypothalamus	0.57±0.14	0.45±0.22	0.29±0.07	0.09±0.08
Thalamus	0.52±0.12	0.44±0.14	0.34±0.08	0.26±0.06
Ventrolateral thalamus	0.42±0.09	0.37±0.12	0.29±0.06	0.26±0.04
Dorsomedial thalamus	0.64±0.21	0.54±0.21	0.44±0.14	0.28±0.09
Pulvinar nucleus	0.62±0.22	0.52±0.17	0.38±0.11	0.25±0.11

Alternate ED_{50} estimates obtained using putamen as a reference region.

Using three parameter fits (i.e., BP_{ND}^{0}, ED_{50} and BP_{ND}^{∞}), global ED_{50} estimates obtained using occipital and putamen as a reference region are very similar (0.14 mg/kg and 0.15 mg/kg, respectively). Based on these three parameter fits, it seems that the non-specific binding (i.e., V_{ND}) in the putamen is intermediate between V_{ND} in the occipital and extra-thalamic regions (i.e., in these regions BP_{ND}^{∞}<0 with putamen and BP_{ND}^{∞}=0 with occipital as a reference region), and V_{ND} in the thalamus (i.e., in this region BP_{ND}^{∞} using occipital > BP_{ND}^{∞} using putamen > 0). Using two parameter fits (BP_{ND}^{0} and ED_{50}), ED_{50} estimates obtained with putamen as the reference region are slightly lower (0.18 mg/kg in thalamus, 0.10 mg/kg globally) than those obtained using occipital as a reference region (0.42 mg/kg in thalamus, 0.16 mg/kg globally).

Table 2: ED_{50} estimates obtained using either putamen or occipital as reference region.

	2-parameter fits		3-parameter fits
Reference region	All regions	Thalamus	All regions
Occipital	0.16 mg/kg	0.42 mg/kg	0.14 mg/kg
Putamen	0.10 mg/kg	0.18 mg/kg	0.15 mg/kg

1. Hilton J, Yokoi F, Dannals RF, Ravert HT, Szabo Z, Wong DF. Column-switching HPLC for the analysis of plasma in PET imaging studies. Nucl Med Biol. 2000 Aug;27(6):627-30.
2. Ichise M, Liow JS, Lu JQ, Takano A, Model K, Toyama H, et al. Linearized reference tissue parametric imaging methods: application to [11C]DASB positron emission tomography studies of the serotonin transporter in human brain. J Cereb Blood Flow Metab. 2003 Sep;23(9):1096-112.