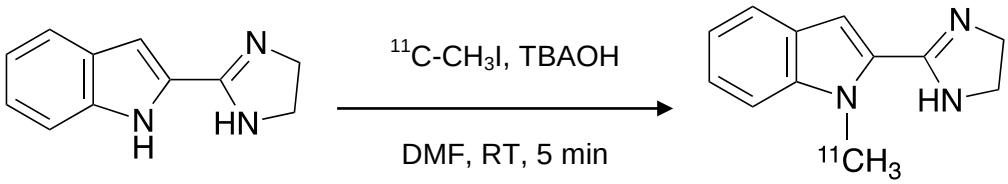
SUPPLEMENTAL MATERIALS AND METHODS

PET Imaging Studies in Rhesus Monkeys

Supplemental Figure 1. ^{11}C-BU99008 synthetic route



BU99007 ^{11}C-BU99008

Radiochemistry. Briefly, ^{11}C-methyl iodide was swept with helium at 18 mL/min through a stainless steel loop pre-loaded with a solution of 0.5 - 0.6 mg BU99007·HCl and tetrabutylammonium hydroxide (1M in MeOH, ~9 µL/mg precursor) in 80 µL of anhydrous *N*,*N*-dimethylformamide (DMF). After the radioactivity peaked in the loop, the reaction mixture was allowed to stand at ambient temperature for 5 min, and injected onto a semi-preparative HPLC system (HPLC column: Agilent SB-Phenyl C-18, 5 µm, 9.4 × 250 mm; Mobile phase: 40 % acetonitrile / 60 % 0.1 M ammonium formate, pH ~10, adjusted with 12 mL of saturated NH_4OH per 1 L of the solvent; Flow rate: 5 mL/min). The product fraction (retention time, ~11 min) was collected, diluted with deionized water (60 mL), and passed through a Waters classic C18 Sep-Pak. The Sep-Pak was rinsed with 0.001 N HCl (10 mL). Product was eluted off the Sep-Pak with absolute ethanol (1 mL), followed by saline (3 mL). The combined EtOH / saline solution was passed through a sterile 0.22 µm membrane filter (Millipore MILLEX MP, 25 mm) for terminal sterilization and collected into a sterile vial containing saline (7 mL) and 40 µL of 4.2 % sodium bicarbonate affording a formulated IV solution ready for dispensing and injection. Purity and specific activity were determined by analytical radio-HPLC coupled with a gamma detector (HPLC column: Phenomenex Prodigy C18 ODS3, 5 µm, 4.6 × 250 mm; Mobile phase: 15 % acetonitrile / 85 % 0.1 M ammonium formate with 0.5 % acetic acid, pH ~4.2; Flow rate: 2 mL/min; UV detector λ = 300 nm).

Study Design.

Supplemental Table 1. Scanning information for each monkey

	Scanning procedure (day, scan order and interval)			
	Day	First	Second	Days between
Monkey 1	1	Baseline	1 mg/kg Moclobemide	N/A
	2	Baseline	0.5 mg/kg Lazabemide	42
	3	Baseline	0.30 mg/kg BU224	24
	4	Baseline	0.03 mg/kg BU224	19
	5	*No Scan	0.01 mg/kg BU224	22
Monkey 2	1	Baseline	1 mg/kg Moclobemide	N/A
	2	Baseline	0.5 mg/kg Lazabemide	29
	3	Baseline	0.03 mg/kg BU224	63
	4	Baseline	0.01 mg/kg BU224	21
	5	Baseline	0.10 mg/kg BU224	27

*There was no scan on the first scan of day 5 for Monkey one. This was due to a failure in the radiosynthesis of the ^{11}C-BU99008.

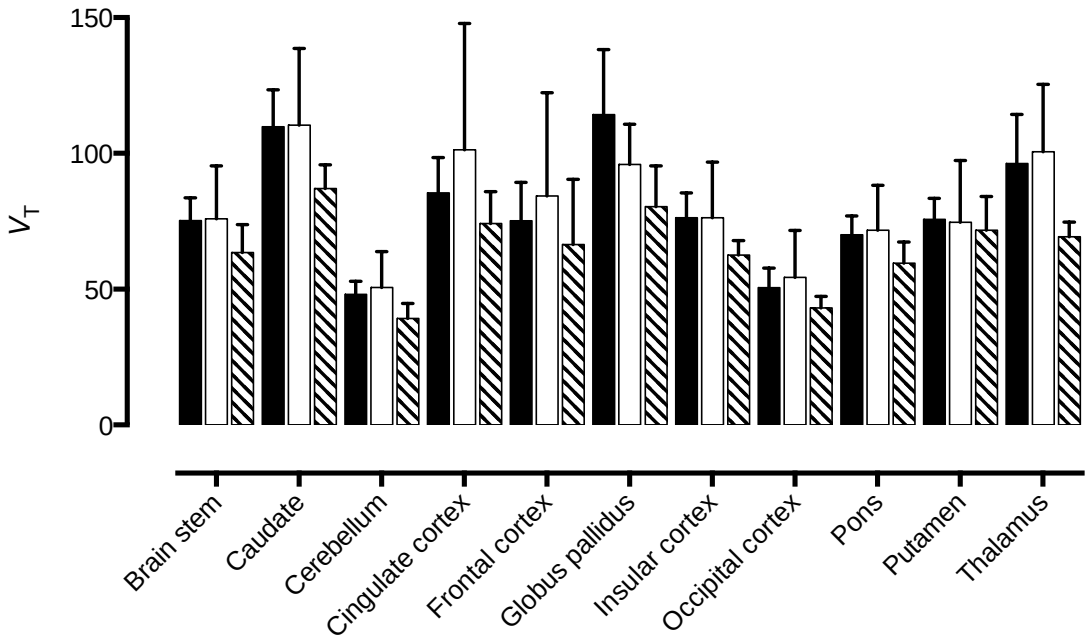
Whole Blood and Plasma Input Functions. Arterial blood samples were collected for the determination of whole blood and plasma input functions of ^{11}C-BU99008. Blood samples were taken at 21 time points following injection of ^{11}C-BU99008. Plasma was separated from red blood cells by centrifugation (2930 *g* for 5 min, 4 °C). Whole blood and plasma samples were counted in a gamma counter (Wizard^2, Perkin Elmer, Waltham, MA, USA).

Metabolite Analysis. Analysis of ^{11}C-BU99008 metabolism in plasma was performed for each scan as follows: arterial plasma samples were collected 5, 15, 30, 60 and 90 min following ligand injection, treated with urea (8 M) and loaded onto a capture column (19 × 4.6 mm) packed with C18 sorbent (Phenomenex SPE Strata-X). The capture column was eluted with 1 % acetonitrile in water at a flow rate of 2 mL/min. After 4 min activity trapped on the capture column was back-flushed off with 21 % acetonitrile and 0.1 M ammonium formate at a flow rate of 1.8 mL.min^{-1} and loaded onto an analytical HPLC column

(Phenomenex Luna (2) C18 5 µm, 4.6 × 250 mm). The HPLC eluent was collected in fractions with an automated fraction collector (Model CF-1, Spectrum Chromatography, Houston, TX) and counted in a gamma counter. The fraction of unchanged radioligand was determined as the sum of the radioactivity in fractions corresponding to ^{11}C-BU99008 (retention time, ~6.5 min) and expressed as percentage of the total amount of radioactivity collected, and fitted to a continuous function. The final plasma input function was calculated as the product of the total plasma curve and the parent fraction curve.

Plasma Free Fraction Measurement. The plasma free fraction (f_p) of ^{11}C-BU99008 was determined using an ultra-filtration-based method. ^{11}C-BU99008 (~250 µCi) in a volume no greater than 0.1 mL was added to 4.0 mL of arterial blood sample taken immediately prior to ligand injection. After 10 min incubation at room temperature, the spiked blood sample was centrifuged (2930 *g* for 5 min) to separate the plasma. A 0.3 mL volume of the spiked plasma was loaded onto the reservoir of the Millipore Centrifree® micropartition device in triplicate and centrifuged at 1228 *g* for 20 min. The f_p was determined by calculating the ratio of the radioactivity in the filtrate to the total activity in the plasma.

Supplemental Figure 2. Bar chart showing the regional distribution volume (V_T) of ^{11}C-BU99008 and the effect of the MAO ligands moclobemide (MAO_A; 1 mg/kg; clear), and lazabemide (MAO_B; 0.5 mg/kg; striped) compared with baseline (black). The bars represent the mean ± SD; V_T generated using the MA1 model.



Supplemental Figure 3. Dose dependant occupancy for the blocking by BU224 in the Globus pallidus (Panel A; ED_{50} = 0.017 mg/kg), Frontal cortex (Panel B; ED_{50} = 0.017 mg/kg) and Cerebellum (Panel C; ED_{50} = 0.016 mg/kg) calculated as a percentage of the baseline scan V_T values for the individual regions after each dose of BU224. V_T data generated using the MA1 model. The fits were performed with a 2-parameter model include ED_{50} and maximum achievable percentage reduction in V_T. Filled symbols: monkey 1, open symbols: monkey 2.