Supplemental Data

Radiosynthesis of ^{11}C-Lu AE92686.

Synthesis of 5,8-dimethyl-2-[2-([^{11}C-1-methyl]-4-phenyl-1*H*-imidazol-2-yl)-ethyl]-[1,2,4]triazolo-[1,5-*a*]pyridine (^{11}C-Lu AE92686) was performed at the PET Centre at Uppsala University by methylation of the precursor 5,8-dimethyl-2-[2-(4-phenyl-1*H*-imidazol-2-yl)-ethyl]-[1,2,4]triazolo[1,5-*a*]pyridine (Fig. 1) with ^{11}C-methyl iodide. The synthesis of the precursor is described in a patent application [Ritzen, A.; Kehler, J.; Langgaard, M. et al. Novel phenylimidazole derivatives as PDE10A enzyme inhibitors for treating neurodegenerative disorder, psychiatric disorder, or drug addiction. 2009, WO-2009152825]. The precursor (2.5 mg) was dissolved in 300 μL of dry dimethylformamide and 2 mg sodium hydroxide was added. ^{11}C-Methyl iodide, prepared according to standard procedures from ^{11}C-CO_2, was introduced and the reaction mixture was heated at 70°C for 90s. The product was purified by semipreparative High-Performance Liquid Chromatography (HPLC). 5 mg ascorbic acid was added to the collected fraction to reduce radiolysis during the work up procedure. Solvent was removed by rotary evaporation and the residue redissolved in 5 mL sterile sodium chloride solution. Identity, radiochemical and chemical purity were assessed with HPLC. For the clinical PET study ^{11}C-Lu AE92686 was produced according to Uppsala PET Centre standard manufacturing procedures, following the Guidelines in the European Pharmacopoeia.

Metabolite Analysis

Rats were injected with ^{11}C-Lu AE92686 and samples from plasma and brain at different time points were analysed by HPLC. Blood samples were centrifuged, plasma separated

and mixed with an equal volume of acetonitrile. The mixture was centrifuged at 16000*g* at 4 °C for 1 min, the supernatant separated and filtered through a 0.2 µm nylon membrane by centrifugation at 16000*g* at 4°C for 1 min. The sample was spiked with Lu AE92686 and separation of the labelled metabolites from ^{11}C-Lu AE92686 was performed by HPLC equipped with a uv-detector on-line with a radioactivity detector. Fractions were collected and the radioactivity in the fractions was measured by a well-type scintillation counter. The fraction containing the ^{11}C-Lu AE92686 was calculated as a percentage of the total radioactivity.

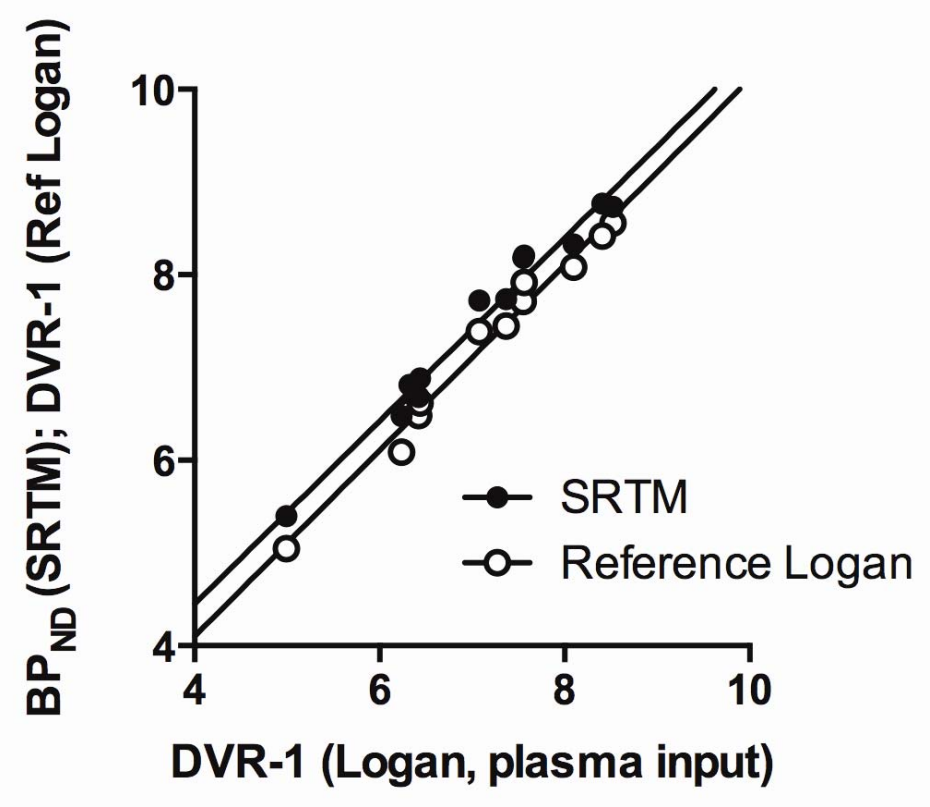
In Vivo Studies in Rodents

Male Sprague Dawley rats (Charles River) weighing 150-225 g, or male NMRI mice (Charles River) weighing 20-28 g, were used. The animals were housed under controlled laboratory conditions (temperature: 21 ± 2°C; humidity: 55 ± 5 %) on a 12:12 h light-dark cycle. Water and food were available ad libitum. *In vivo* binding studies were performed as described in Larsen *et al.* [Larsen, A.K.; Brennum, L.T.; Egebjerg, J. et al. Selectivity of ^{3}H-MADAM binding to 5-hydroxytryptamine transporters in vitro and in vivo in mice; correlation with behavioural effects. Br J Pharmacol. 2004;141:1015-1023]. Rats or mice were injected with 100-300 kBq ^{3}H-Lu AE92686 intravenously (i.v.) via the tail vein. Five to twenty minutes after injection of the radioligand the animals were sacrificed and decapitated. The brain was quickly removed and striatum and cerebellum were dissected out. The tissues were homogenised in ice cold buffer (50 mM K_2PO_4, pH 7.4) and were immediately filtered through 0.1% polyethylenimine (PEI) soaked WhatmanGF/C filters. Filters were washed two times with ice cold buffer and counted in a scintillation counter. For displacement studies, animals were pre-treated with the PDE10 inhibitor 2-[4-(1-methyl-4-pyridin-4-yl-

1H-pyrazol-3-yl)-phenoxymethyl]-quinoline (MP-10) administered subcutaneously (s.c.) at 6 different doses (dose range 0.01-1.0 mg/kg) 30 or 60 min before decapitation (6-12 animals/dose). Vehicle treated animals were used to determine total binding. Brain homogenate was measured for protein content by the bicinchoninic acid (BCA) protein determination assay [Smith, P.K.; Goeke, N.M.; Olson, B.J.; Klenk, D.C. Measurement of protein using bicinchoninic acid. Ann Biochem. 1985;150:76-85]. *In vivo* ED_{50} values were determined using non-linear regression.

Determination of Free Fraction in Plasma.

Before injection of the tracer, ~1.5 mL of blood was collected in tubes with sodium heparin (Vacuette, Greiner Bio-One GmbH) and mixed with 10 µL of the tracer. The tubes were then centrifuged at 3,000*g* for 4 min at +4 °C to obtain plasma. Plasma (3 x 200 µL) was then transferred to 3 ultracentrifugation units (Microcon, Ultracel YM-30, Milipore, Billerica, MA, USA), which were centrifuged at 20,800*g* for 12 min at +4 °C. Finally, 50 µL of the resulting filtrates were transferred to new tubes and radioactivity was measured. Additionally, radioactivity was also measured in 50 µL (triplicate) of the original plasma samples. For compensation of potential loses of radioligand adsorbed to plastic surfaces or filter membranes, 10 µL of tracer was mixed to a volume of buffer (50 mM Tris-HCl, pH 7.4) equal to the volume of blood used above, and the same procedure was performed, as described above. The fraction of free tracer in plasma was defined as the ratio between radioactivity in filtrate and that in plasma, divided by the ratio between radioactivity in filtrate and that in buffer.



Supplemental Figure 1. Graphical Logan plot. BP_{ND} based on SRTM (closed circles) and DVR-1 based on the reference Logan method (open circles) versus DVR-1 based on Logan with plasma input function. The solid lines are Deming regressions.