Supporting Information

Positron Emission Tomography Imaging Evaluation of a Novel ^{18}F-Labeled Sigma-1 Receptor Radioligand in Cynomolgus Monkeys

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1. Instrumentation for radiochemistry procedures

Instrumentation for radiochemistry procedures and the production of ^{18}F-fluoride have been described previously.[1] $H_2{}^{18}O$ was obtained from Huayi Isotopes (Toronto, Canada). Anion exchange Chromafix cartridges (PS-HCO_3) for ^{18}F-fluoride trapping were purchased from Macherey-Nagel (Dueringen, Germany). Solid-phase extraction (SPE) cartridges were purchased from Waters Associates (Milford, MA, USA).

A preparative HPLC system used for purification of the radiotracer included a Shimadzu LC-20A pump, a Rheodyne 7133i injector with a 2 mL loop, a Knauer K200 ultraviolet detector, a Bioscan γ-flow detector, and a laptop computer running the EZStart data acquisition software. An analytical HPLC system included a Shimadzu LC-20A quaternary pump, a Rheodyne 7133i injector with a 100 μL loop, a Shimadzu SPD-M20A PDA or SPD-20A ultraviolet detector, a Bioscan flow cell γ-detector, and a PC with Shimadzu Class VP 7.2 software for system control and data acquisition.

2. Procedures for PET imaging

PET imaging experiments were performed in cynomolgus monkeys according to a protocol approved by the Yale University Institutional Animal Care and Use Committee, as described previously.[1] Two 7-year old female monkeys (3.8 and 4.7 kg) were used in this study. The animals were immobilized with ketamine (10 mg/kg intramuscularly) and anesthetized with 1.5%–2.5% isoflurane. An arterial line was placed in the radial or femoral artery for blood sampling. Scans were acquired on a FOCUS 220 scanner (Siemens Medical Solutions, Knoxville, TN, USA). Before radioligand injection, a 9-min transmission scan was obtained for attenuation correction. Baseline scans were obtained over 3 h or 2 h to assess kinetic and binding profiles. The tracer was injected intravenously over 3 min as a slow bolus (121.4 and 74.7 MBq, respectively, in 10 mL). Two-hour blocking scans of [^{18}F]FBFP were acquired with the σ_1 receptor agonist SA4503 [2] at 0.5 mg/kg dose given intravenously as a 3-min slow bolus injection at 10 min before radioligand administration (59.3 and 150.6 MBq in 10 mL) to evaluate *in vivo* binding specificity. A total of four PET scans were obtained.

3. Procedures for plasma metabolite analysis and arterial input function measurement

Measurement of the arterial input function, including sample preparation, metabolite analysis, and data processing, have been described previously.[1] Arterial blood samples were collected at pre-selected time points and the radioactivity concentrations in the whole blood and plasma were measured with γ-counters (Wizard

1480/2480, PerkinElmer, Waltham, MA, USA). During each scan, blood samples taken at 3, 8, 15, 30, 60, 90 and 120 min (and one more time point at 180 min for the 3 h baseline scan), after injection were processed and analyzed by HPLC using a modified column-switching system[3] to determine the fraction of unmetabolized tracer over the course of the scan. The unmetabolized parent fraction was determined as the ratio of the sum of radioactivity in fractions containing the parent compound to the total amount of radioactivity collected. A biexponential function was fitted to the measured parent fractions to produce a continuous function describing the parent fraction over time. The arterial plasma input function (AIF) was calculated as the product of the total counts in the plasma and the interpolated parent fraction at each time point. The measured input function values were fitted to a sum of 3 exponentials, and the fitted values were used as inputs for kinetic analyses.

Procedures for blood sample processing and HPLC analysis are as follows. Blood samples in ethylenediaminetetraacetic (EDTA) tubes were centrifuged at 2,300 g at 4 °C for 5 min to separate the plasma. Plasma samples were treated with urea (8 M) and loaded onto a capture column (19 x 4.6 mm) packed with Phenomenex SPE C18 Strata-X sorbent and eluted with 1% acetonitrile in water at a flow rate of 2 mL/min for 4 min, after which the trapped activity was backwashed onto a Synergi RP or Gemini NX column (5 μm, 4.6 mm x 250 mm) and eluted with 40% acetonitrile/ 60% ammonium formate (0.1 M, pH = 6.4) at a flow rate of 1.15 or 1.2 mL/min. The HPLC eluent was collected by an automated fraction collector (model CF-1; Spectrum Chromatography, Houston, TX), and the fractions were counted in a γ-counter.

4. Procedures for image analysis and kinetic modeling

Procedures for PET image reconstruction, definition of regions of interest (ROIs), and kinetic analysis have been detailed previously.[1] High-resolution magnetic resonance (MR) images were acquired with a Siemens 3T Trio scanner to assist with image co-registration and anatomical localization of regions of interest (ROIs). The MR image was registered to an atlas and to the PET images.

PET emission data were attenuation-corrected using the transmission scan, and dynamic images were reconstructed using a Fourier rebinning and filtered back projection algorithm with a Shepp-Logan filter. ROIs were defined from a single representative anatomic cynomolgus MR image registered to a template image. Registration parameters were derived to apply ROIs to each PET scan, and time–activity curves (TACs) were generated for the following 15 brain regions: amygdala, brainstem, caudate, cerebellum, cingulate cortex, frontal cortex, globus pallidus, hippocampus, insula, occipital cortex, parietal cortex, putamen, substantia nigra,

temporal cortex, and thalamus.

5. Supporting Figures

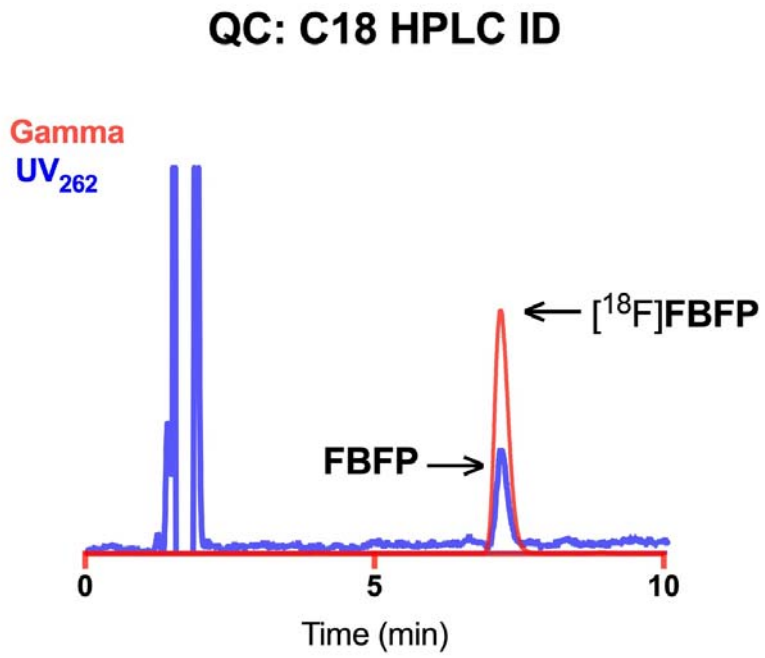

Figure S1. HPLC co-elution profiles of FBFP (**8**) and [^{18}F]FBFP ([^{18}F]**8**), with retention times of 6.97 and 7.03 min, respectively. Condition: 17% acetonitrile/ 83% 0.1 M ammonium formate with 0.5% acetic acid (pH = 4.2), flow rate = 2 mL/min.

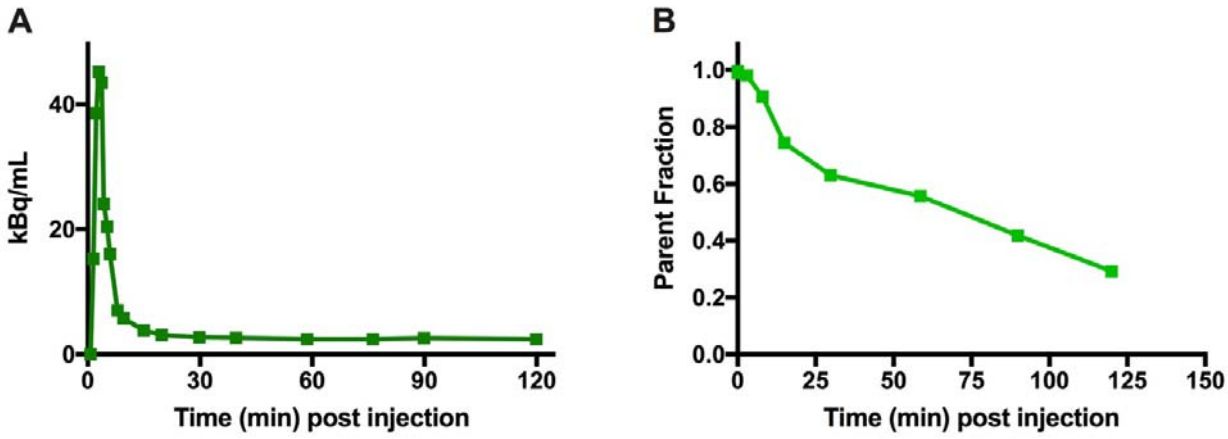

Figure S2. Plasma analysis of [^{18}F]FBFP ([^{18}F]**8**): Total radioactivity concentration (**A**) in plasma and parent fraction (**B**) over time, from the baseline scan in monkey 2.

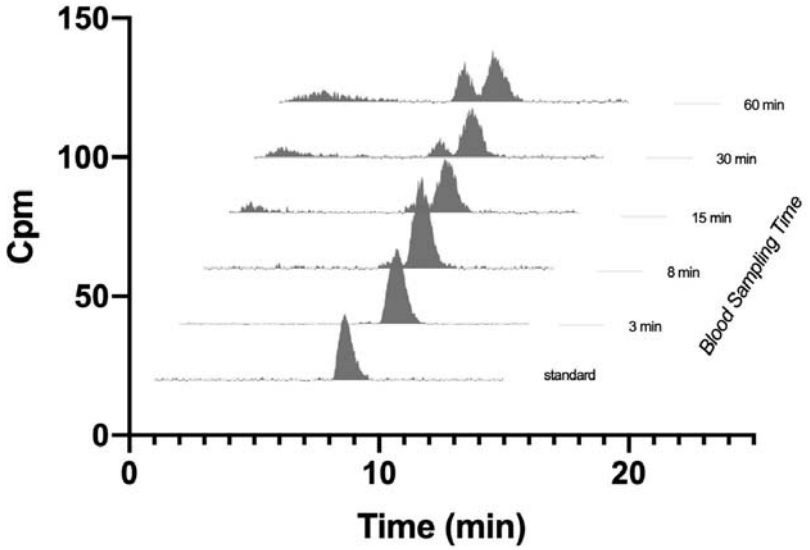

Figure S3. Time course for [^{18}F]FBFP ([^{18}F]**8**) and its radioactive metabolites in plasma, showing gamma HPLC chromatograms from analysis of plasma samples at different time points after injection from a baseline scan in monkey 2 using Gemini NX column. The peak at 8-9 min is the parent compound.

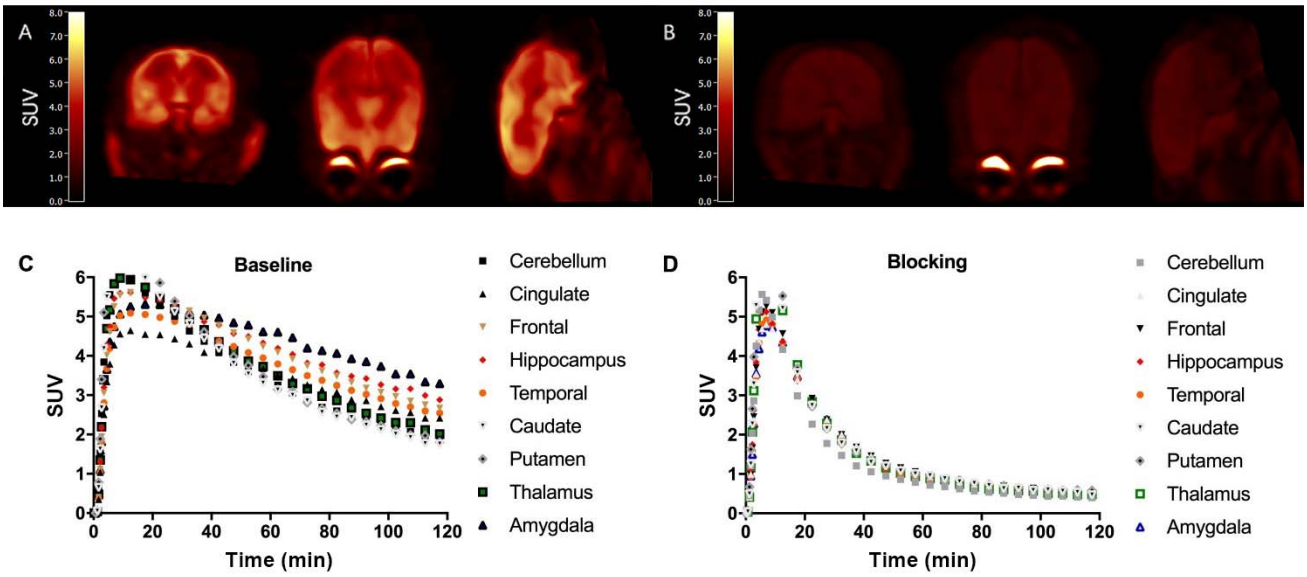

Figure S4. PET images (coronal, transverse, and sagittal views) and regional time-activity curves of [^{18}F]FBFP ([^{18}F]**8**) from the baseline and blocking scans in monkey 2. PET Images are summed from 30-45 min after [^{18}F]FBFP injection. SUV = standardized uptake value. For clarity, not all brain regions are displayed.

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