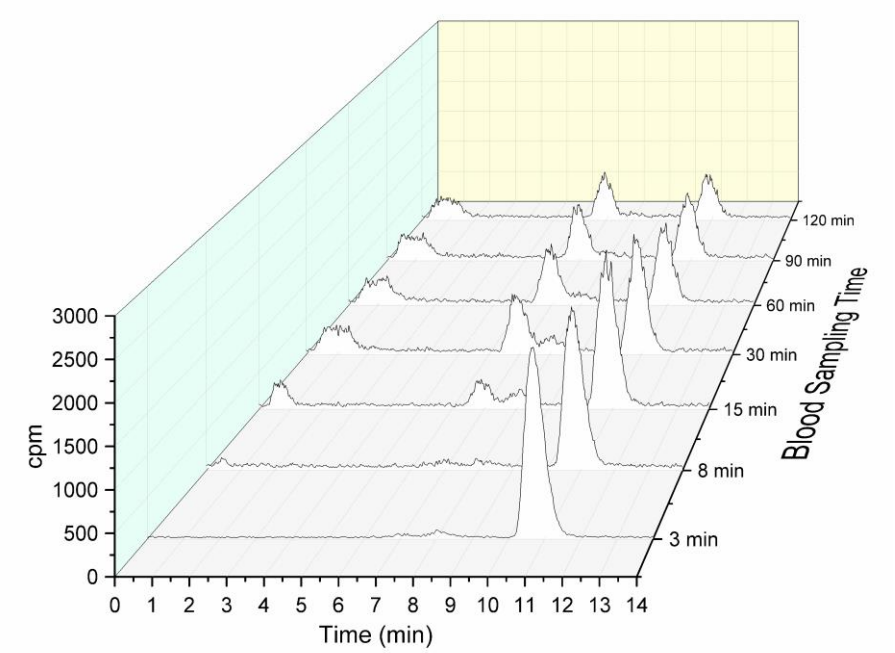
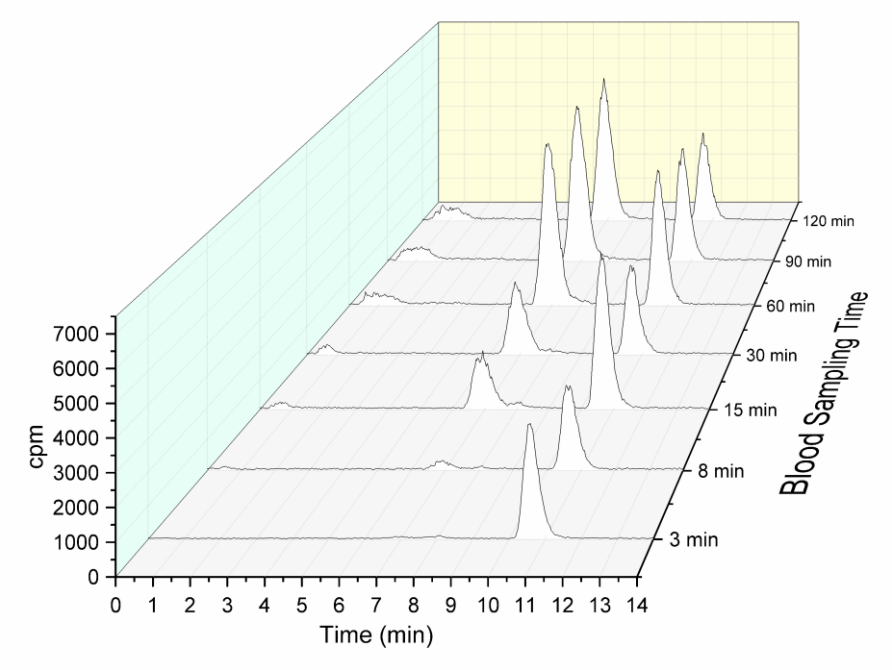
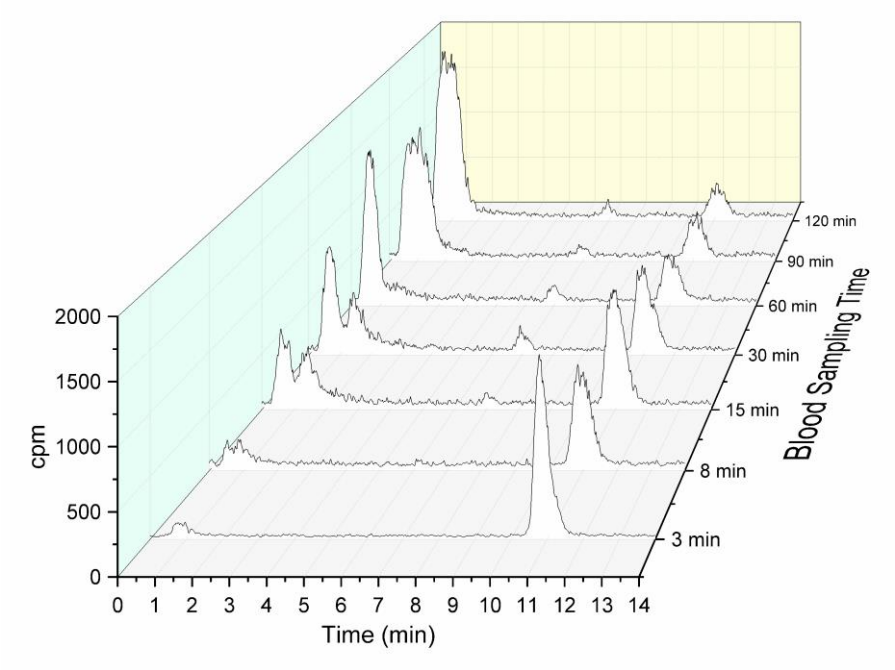
Evaluation of Four Sigma-1 PET Tracers: Supplemental



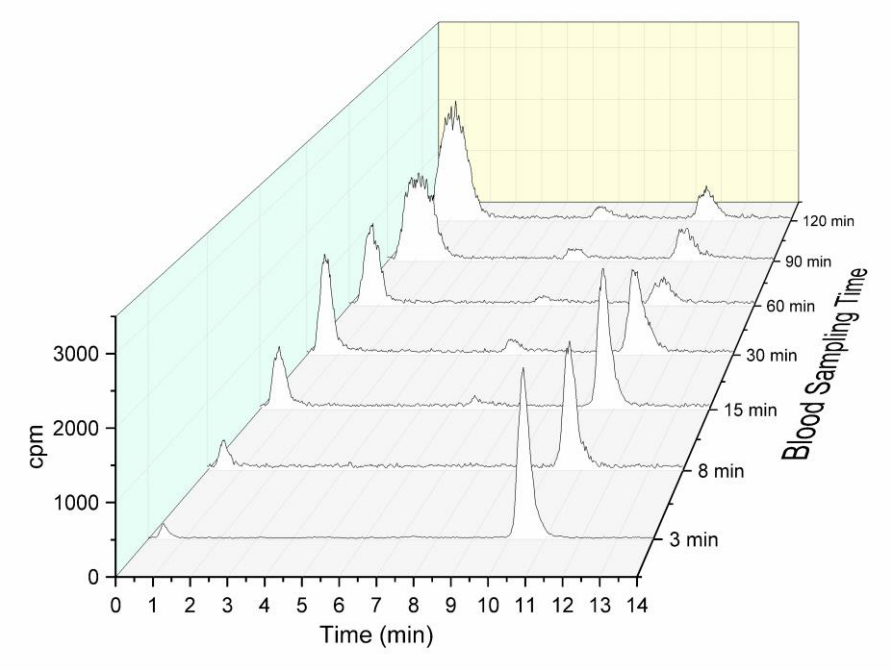
Supplemental Figure 1. Time course for metabolism of ^{18}F-1 in plasma, showing gamma HPLC chromatograms. The peak at ~11 min is the parent compound, and the rest are metabolites. HPLC conditions: Contents on the capture column were backwashed onto a Phenomenex Luna C18 (2) column (5 µm, 4.6 x 250 mm) eluting with 60% acetonitrile/40% 0.1 M ammonium formate, flow rate = 1.20 mL/min.



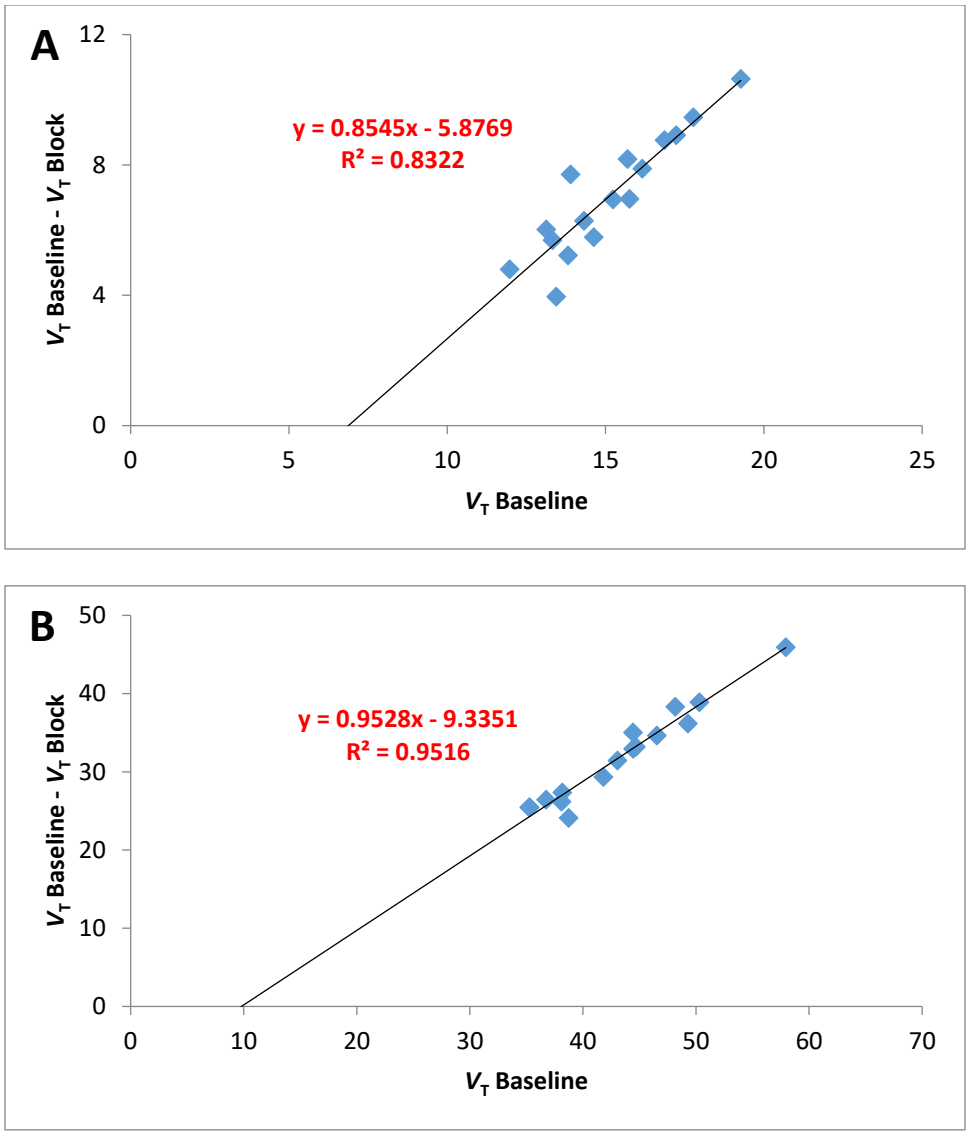
Supplemental Figure 2. Time course for metabolism of ^{18}F-2 in plasma, showing gamma HPLC chromatograms. The peak at ~11 min is the parent compound, and the rest are metabolites. HPLC conditions: Contents on the capture column were backwashed onto a Phenomenex Luna C18 (2) column (5 µm, 4.6 x 250 mm) eluting with 60% acetonitrile/40% 0.1 M ammonium formate, flow rate = 1.20 mL/min.



Supplemental Figure 3. Time course for metabolism of ^{18}F-3 in plasma, showing gamma HPLC chromatograms. The peak at ~11 min is the parent compound, and the rest are metabolites. HPLC conditions: Contents on the capture column were backwashed onto a Phenomenex Luna C18 (2) column (5 µm, 4.6 x 250 mm) eluting with 45% acetonitrile/55% 0.1 M ammonium formate, flow rate = 1.35 mL/min.



Supplemental Figure 4. Time course for metabolism of ^{18}F-4 in plasma, showing gamma HPLC chromatograms. The peak at ~10.5 min is the parent compound, and the rest are metabolites. HPLC conditions: Contents on the capture column were backwashed onto a Phenomenex Luna C18 (2) column (5 µm, 4.6 x 250 mm) eluting with 40% acetonitrile/60% 0.1 M ammonium formate, flow rate = 1.50 mL/min.



Supplemental Figure 5. Receptor occupancy plots for blocking of ^{18}F-2 and ^{18}F-4 binding with 0.5 mg/kg of SA4503 in the same adult rhesus monkey. Plot of changes in 1T derived V_T between baseline and 120 min blocking scans vs. 240 min baseline scans for sixteen brain regions for ^{18}F-2 (A) and ^{18}F-4 (B). The slope of the regression is the estimated S1R occupancy by SA4503, and the x-intercept is the estimated nondisplaceable volume of distribution (V_{ND}) from which BP_{ND} can be calculated.

Association and dissociation studies of ^{18}F-1 and ^{18}F-2

Methods

Kinetic studies with rat and human sigma-1 receptors were performed using membrane homogenates obtained from the rat cortex (female SPRD rats, 10-12 weeks old) and HEK293 cells stably transfected with human SIGMAR1 (by courtesy of Olivier Soriani, Institute of Biology Valrose, UNS Université Nice Sophia Antipolis, France), respectively, as well as identical batch of the respective radioligand ^{18}F-1 [(R)-^{18}F-fluspidine] or ^{18}F-2 [(S)-^{18}F-fluspidine]. Association and dissociation experiments were conducted at room temperature in incubation buffer (50 mM TRIS, pH 7.4, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$). Non-specific binding was determined by addition of 1 µM haloperidol in the incubation buffer.

Association studies were started with the application of radioligand, and receptor-bound ligand separated from free ligand by filtration (GF-B glass-fibre filter; 48-sample harvester, Brandel, Gaithersburg, MD, USA) after 0.5, 1, 3, 5, 7, 10, 15, 20, 30, 45, 60, 90, 120, and 180 min incubation.

For dissociation studies, receptor preparation and ^{18}F-1 or ^{18}F-2 were pre-incubated for 60 min or 180 min, respectively. Dissociation was started by the addition of 30 µM unlabeled 1 or 2 in the incubation buffer. Samples were taken at 0.5, 1, 3, 5, 10, 20, 30, 45, and 60 min after incubation with ^{18}F-1 or^{18}F-2, and additional samples were taken at 90, 120, and 180 min of incubation with ^{18}F-1. Receptor-bound ligand was separated from free ligand by filtration as described above. Filter-bound radioactivity was measured by gamma-counting (Wallac Wizard 1480, PerkinElmer, Rodgau, Germany) and specific binding at various times calculated.

By non-linear regression analyses (GraphPad Prism 3.0, GraphPad Software Inc., La Jolla, CA, USA), the observed rate constant k_{obs} and the dissociation rate constant k_{off} were calculated from the association and dissociation experiments, respectively. The association rate constant k_{on} was calculated according to $k_{on} = (k_{obs}-k_{off})/[radioligand]$, and the K_D value according to $K_D = k_{off}/k_{on}$. Association half-time (Ass. $t_{1/2}$) and dissociation half-time (Diss. $t_{1/2}$) were also calculated.

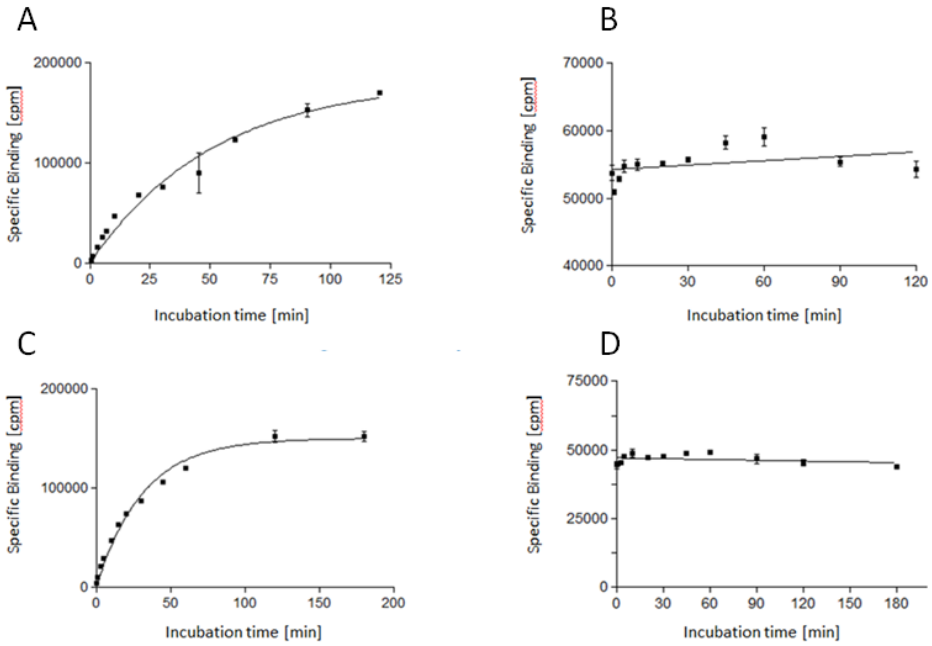
Results

The results from kinetic studies of ^{18}F-1 and ^{18}F-2 are presented in Supplemental Figures 6 and 7. Supplemental Table 1 lists the calculated kinetic parameters for ^{18}F-1 and ^{18}F-2. The dissociation rate for ^{18}F-1 was extremely low and could not be reliably measured. As a result, the dissociation constant K_D could not be determined for ^{18}F-1.

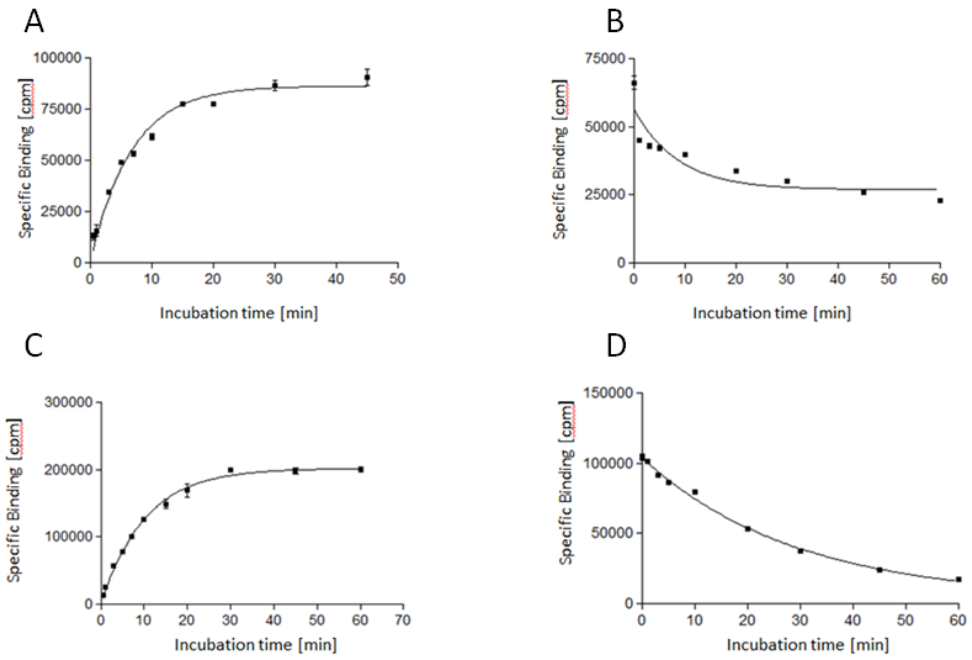
Supplemental Table 1: In vitro kinetic parameters of ^{18}F-1 and ^{18}F-2

Radioligand	**σ_1 receptor type**	**k_{on} ($M^{-1}min^{-1}$)**	**k_{off} (min^{-1})**	**K_D (nM)**	**Ass. $t_{1/2}$ (min)**	**Diss. $t_{1/2}$ (min)**
^{18}F-1	human	0.0318	n.d	n.d	22	> 120
	rat	0.0318	n.d	n.d.	47	> 180
^{18}F-2	human	3.46×10^8	0.0342	0.099	7.1	20.3
	rat	5.83×10^8	0.1213	0.208	3.9	5.7

n.d. = non-determinable



Supplemental Figure 6. Association (A, C) and dissociation (B, D) rate measurements of ^{8}F-1 in rat cortex homogenates (A, B) and cloned human sigma-1 receptor (C, D).



Supplemental Figure 7. Association (A, C) and dissociation (B, D) rate measurements of ^{18}F-2 in rat cortex homogenates (A, B) and cloned human sigma-1 receptor (C, D).