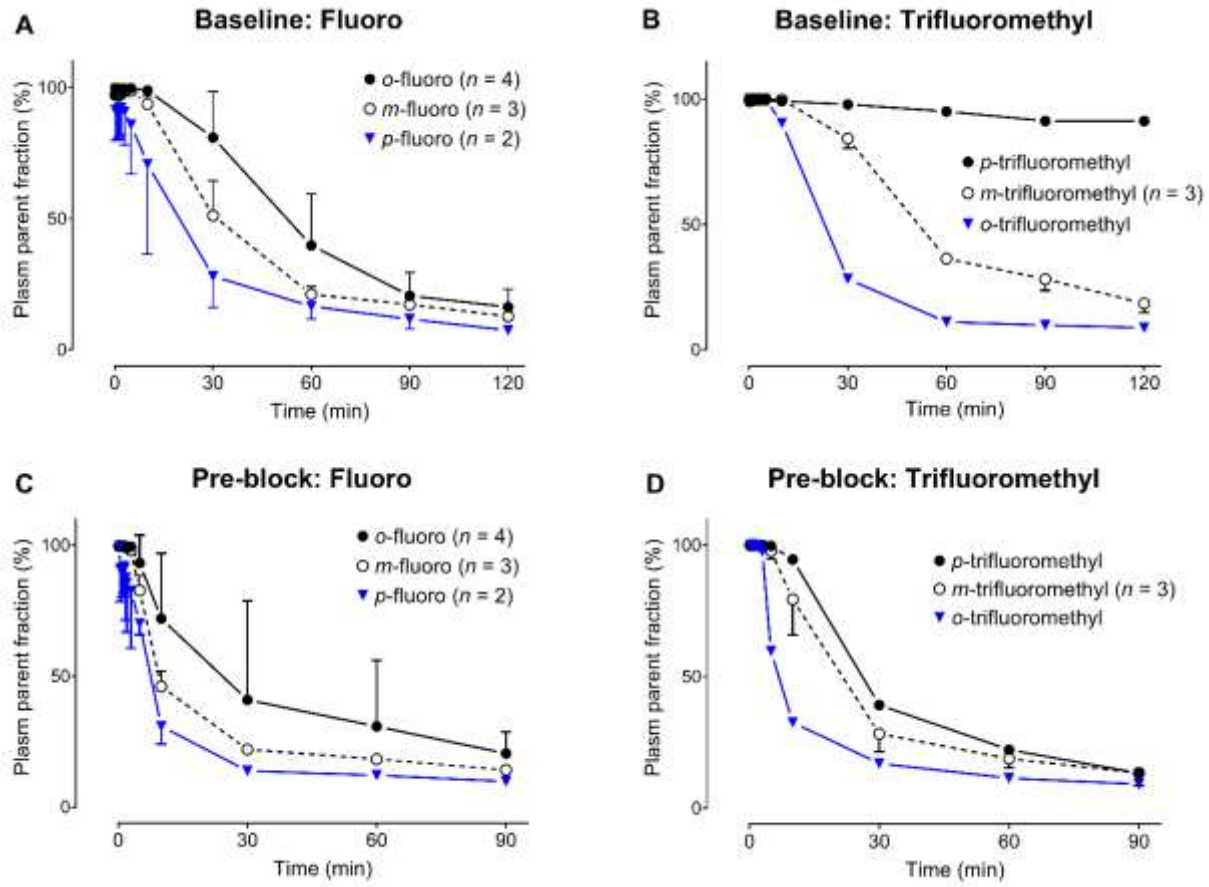
Kinetic Analysis

All kinetic analyses were performed using PMOD 3.9 (PMOD Technologies Ltd., Zurich, Switzerland). PET images were coregistered to a standardized monkey magnetic resonance imaging template (*1*). Thirty-four predefined brain regions of interest from the template were applied to the coregistered PET image to obtain regional time-activity curves. Brain uptake was expressed as a standardized uptake value (SUV), which normalizes for injected radioactivity and body weight.

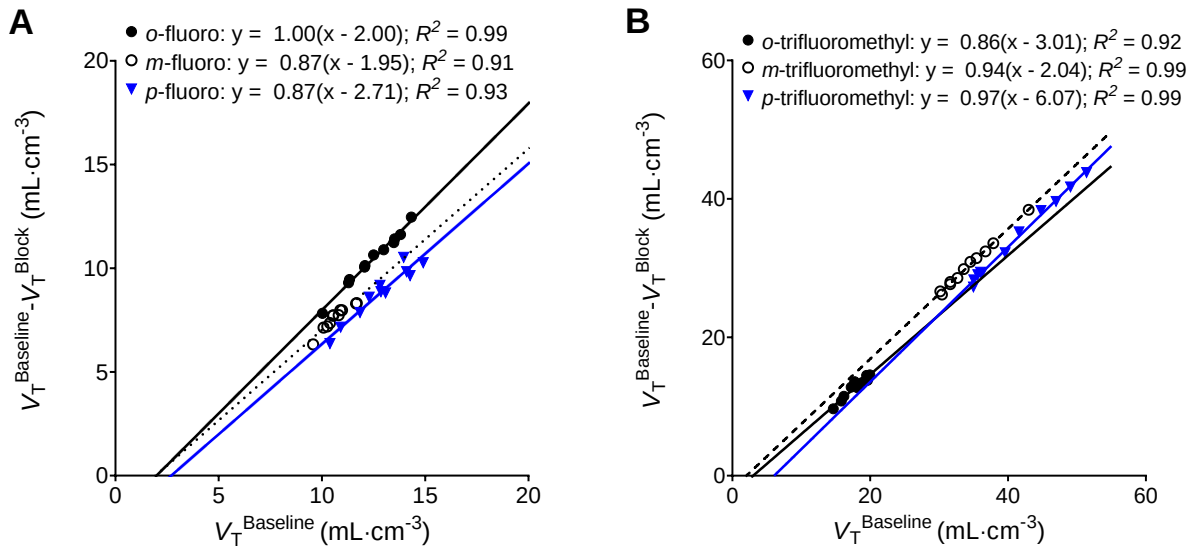
Distribution volume (V_T) was estimated for different regions. For a more robust estimation of V_T, Logan graphical analysis was used and applied to compare performance across all radioligands. For both baseline and pre-blocked conditions, only 90 minutes of brain time-activity curves and the radiometabolite-corrected arterial input function were used. Starting PET frames used for regression were selected based on the equilibration time (t^*) of the whole brain. The equilibration time was automatically determined for the maximum allowed regression error of 10%. The equilibration time was similar for the six radioligands and generally ranged between 10 and 20 minutes. The identifiability of V_T (i.e., percent standard error estimated from the theoretical parameter covariance matrix) was also determined. Parametric images of brain TSPO binding were generated for visual comparison of brain uptake among radioligands as well as between baseline and pre-blocked studies.

SUPPLEMENTAL REFERENCES

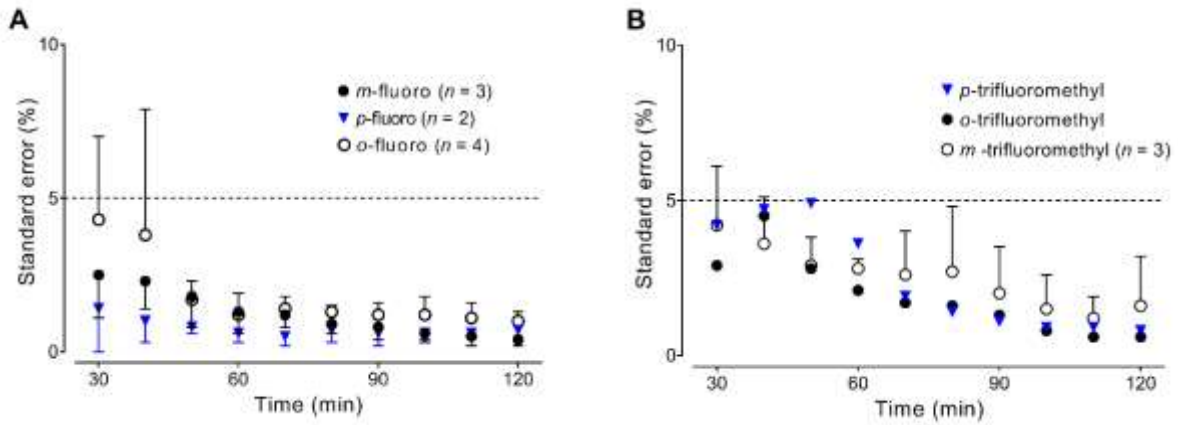
1. Yasuno F, Brown AK, Zoghbi SS, et al. The PET radioligand [11C]MePPEP binds reversibly and with high specific signal to cannabinoid CB1 receptors in nonhuman primate brain. *Neuropsychopharmacology.* 2008;33:259-269.



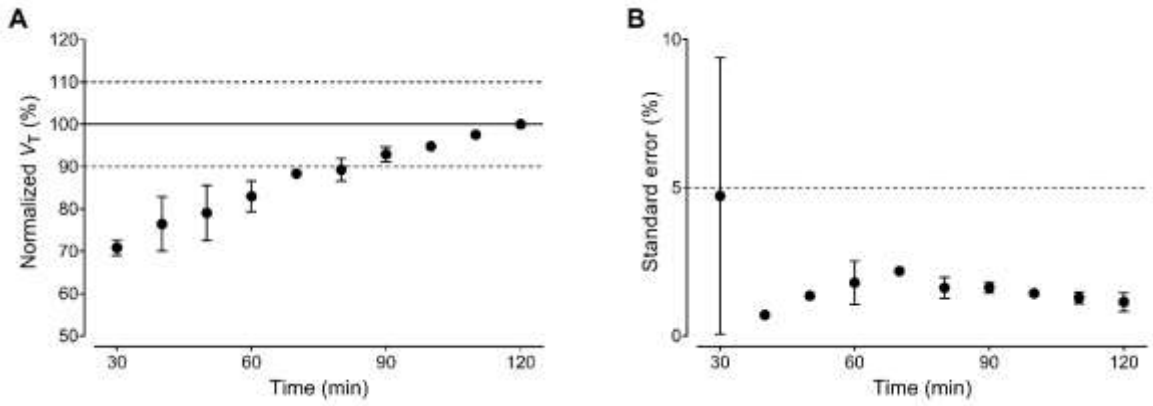
Supplemental Figure 1. Time course of parent fraction in plasma at baseline and for pre-blocked scans for the fluoro (A, C) and trifluoromethyl (B, D) radioligands.



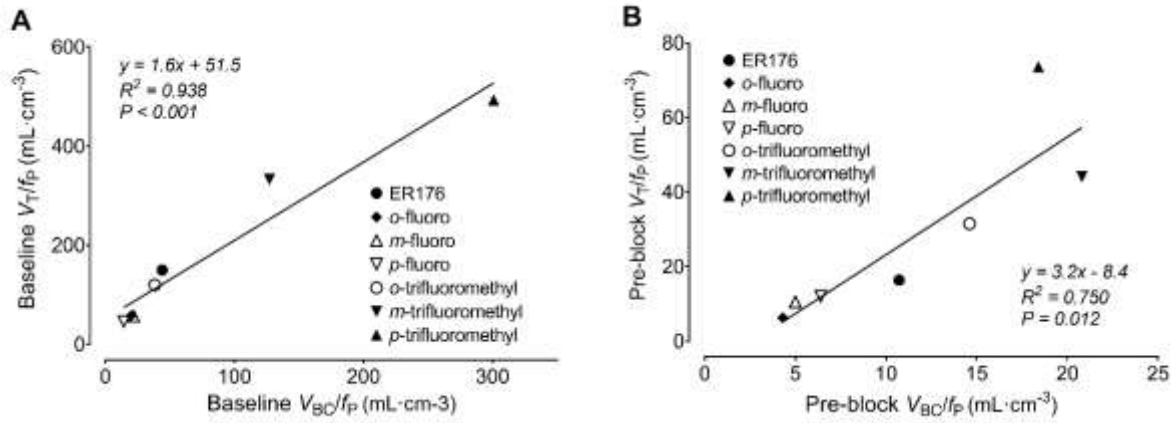
Supplemental Figure 2. Lassen plot to determine receptor occupancy and nondisplaceable distribution volume (V_{ND}/f_P) of the fluoro (A) and trifluoromethyl (B) radioligands in monkey brain. One representative data point is plotted for the radioligands with multiple replication studies.



Supplemental Figure 3. Identifiability of whole-brain total distribution volume (V_T) for the fluoro (A) and trifluoromethyl (B) radioligands. V_T was calculated via Logan graphical analysis and normalized to the terminal V_T value at 120 minutes. Points represent the mean normalized V_T (± SD).



Supplemental Figure 4. Time-stability analysis (A) and identifiability (B) of whole-brain total distribution volume (V_T) for ^{11}C-ER176. V_T was calculated via Logan graphical analysis and normalized to the terminal V_T value at 120 minutes. Points represent the mean normalized V_T (± SD).



Supplemental Figure 5. The correlation between distribution volume in blood cells corrected for plasma free fraction (V_{BC}/f_P) and whole-brain distribution volume (V_T/f_P) for baseline (A) and pre-blocked (B) scans.

Supplemental Table 1. Summary of experimental protocols for six fluorine-containing analogs of ^{11}C-ER176

	Reference	Fluoro			Trifluoromethyl		
Radioligand	^{11}C-ER176	*o*-fluoro	*m*-fluoro	*p*-fluoro	*o*-trifluoromethyl	*m*-trifluoromethyl	*p*-trifluoromethyl
No. of studies	2	4	3	2	1	3	1
Weight (kg)	11.1	12.9	11.3	12.8	13.9	12.3	14.4
Injected activity (MBq)	246	241	300	274	272	242	269
Molar activity (MBq/nmol)	107	132	341	223	88	87	204
Injected mass (nmol/kg)	0.29	0.16	0.10	0.16	0.22	0.40	0.09

Data are presented as mean.

Supplemental Table 2. Comparison of total distribution volume (V_T), occupancy, nondisplaceable distribution volume (V_{ND}), and nondisplaceable binding potential (BP_{ND}) of the whole brain among ^{11}C-ER176 and six fluorine-containing analogs.

	V_T (mL·cm^{-3})						
	Baseline	Pre-block	Blockade (%)	Occupancy (%)	V_{ND} (mL·cm^{-3})	BP_{ND}	Binding affinity ratio[a]
Reference							
^{11}C-ER176	20.5	2.9	85.4	98.3	2.5	6.8	1.3
Fluoro							
o-fluoro	18.9	3.4	81.1	91.9	2.0	9.6	2.8
m-fluoro	17.9	3.3	78.1	90.0	2.6	5.9	2.7
p-fluoro	12.2	3.8	69.2	87.0	2.7	3.7	2.9
Trifluoromethyl							
o-trifluoromethyl	17.8	5.1	71.4	86.0	3.0	4.9	5.5
m-trifluoromethyl	35.7	4.3	87.1	95.3	2.9	11.6	2.0
p-trifluoromethyl	41.0	7.0	82.6	97.3	6.1	5.7	0.8

Data are presented as mean.

[a] Ratio of binding affinity (K_i in nM) in low-affinity binders to that of high-affinity binders.