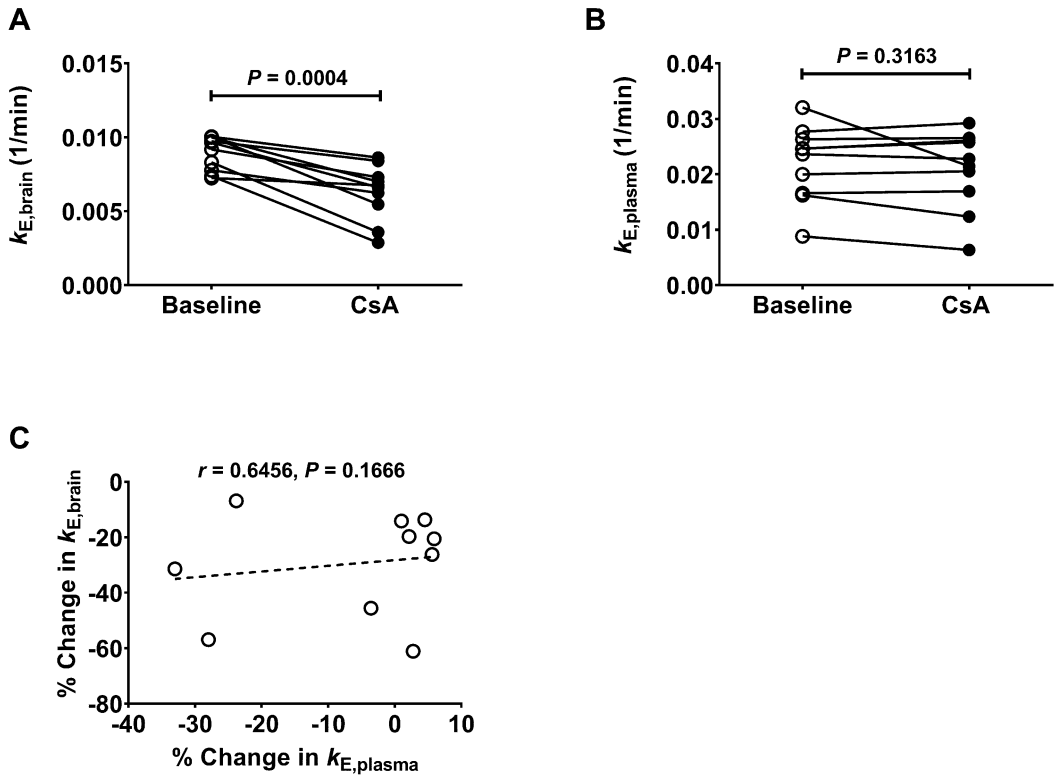
Determination of Cyclosporine A Concentrations in Blood

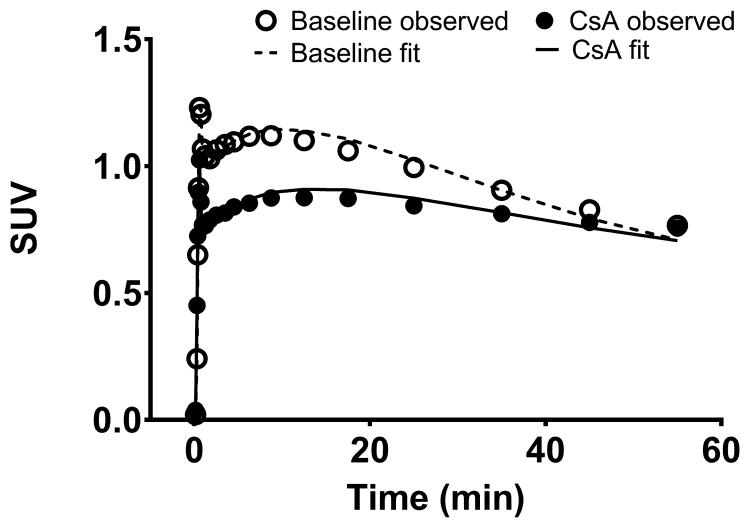
The concentration of cyclosporine A in whole blood was determined by high-performance liquid chromatography (HPLC) with minor modifications as described previously (*1*). Briefly, after hemolysis of 1 mL whole blood by the addition of a mixture of zinc sulfate/methanol (65:35, w/v; 2 mL), 50 µL (1.0 µg) of cyclosporine D (Cayman Chemical, Ann Arbor, MI, USA) was added as the internal standard. After centrifugation (5 min, 3,000 x g), the supernatant was passed through an Oasis HLB 1 cc SPE cartridge (30 mg; Waters Corporation, Milford, MA, USA) which had been equilibrated with 2 mL of methanol and water (pH 3.0), respectively. The column was washed with methanol in water (50%, v/v; 2.3 mL) and heptane (0.5 mL), and cyclosporine A was eluted with ethanol (100%, 300 µL). The eluate was mixed with 100 µL of water (pH 3.0) and 1 mL of heptane and centrifuged for 5 min at 3,000 x g. An aliquot (80 µL) of the aqueous phase was injected onto the HPLC column. HPLC was performed using a Dionex "UltiMate 3000" system (Dionex Corp., Sunnyvale, CA) with UV detection at 205 nm. Chromatographic separation was carried out at 75°C on a Hypersil BDS-C18 column (5 µm, 250 x 4.6 mm, Thermo Fisher Scientific, Inc, Waltham, MA), preceded by a Hypersil BDS-C18 pre-column (5 µm, 10 x 4.6 mm). The mobile phase consisted of a continuous gradient, mixed from solvent A (acetonitrile in water [35:75, v/v]) and solvent B (acetonitrile in water [85:15, v/v]). The column was equilibrated with 45% solvent B at time 0; after injection of the sample (80 µL), the content of solvent B was linearly increased to 93% at 19 min. Subsequently, the percentage of solvent B was decreased to 45% within 2 min, to equilibrate the column for 8 min before application of the next sample. Linearity was tested by assaying drug-free whole blood spiked with 0.1, 0.5, 2.5 and 10 µg cyclosporine A. The calibration curve for cyclosporine A in blood was linear over the tested concentration range (correlation coefficient: 0.985). Quantification of cyclosporine A was based on the comparison of cyclosporine A/cyclosporine D ratios.

Determination of Parent ^{11}C-metoclopramide in Plasma

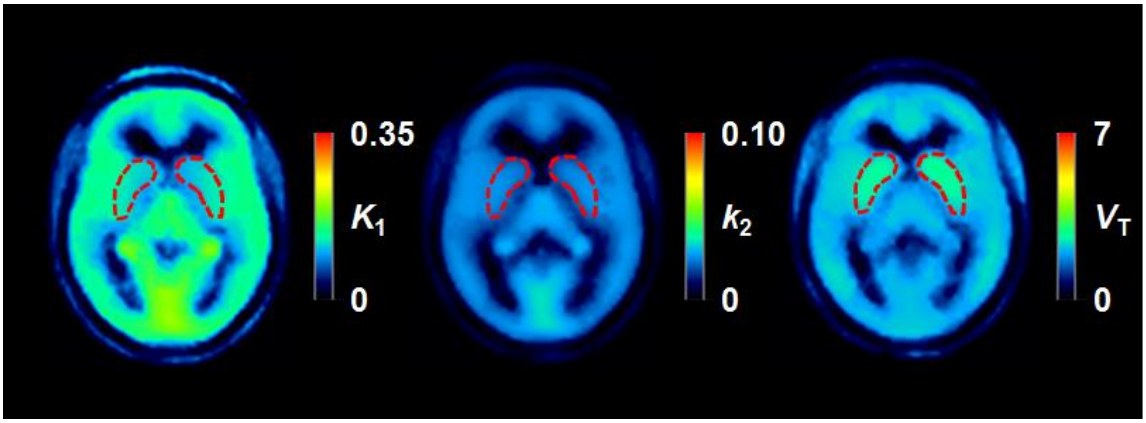
Plasma samples (830 μL) collected at 5, 10, 20, 30 and 40 min after ^{11}C-metoclopramide injection were mixed with acetonitrile (600 μL) and vortexed to precipitate plasma proteins. After addition of water (600 μL) and phosphate-buffered saline (10-fold concentrate, pH 7.4, 100 μL) samples were centrifuged (4 min, 15,000 x g, 4°C) and the protein pellet and supernatant were separately counted in a gamma counter to determine the recovery of radioactivity, which was for the 10 min time point 87 ± 2% for the baseline scan and 88 ± 2% for the CsA scan (n = 9). The supernatant (1.5 mL) was then injected into the HPLC system. An Atlantis T3 OBD HPLC column (250 x 10 mm, 10 μm, Waters, Austria) equipped with a pre-column (Atlantis T3 Prep Guard Cartridge, 10 x 10 mm, 10 μm) was eluted with a mixture of 25 mM aqueous ammonium acetate (solvent A) and acetonitrile (solvent B). A linear gradient from 20% to 30% of solvent B over 5.5 min (total run length: 10 min) was applied to the column at a flow rate of 5 mL/min. On this HPLC system ^{11}C-metoclopramide and its radiolabeled metabolites eluted with retention times of approximately 8.5 and 4 min, respectively. HPLC eluates were collected in 1-min fractions, which were counted for radioactivity in a gamma counter. The measured fractions were corrected for radioactive decay to determine the percentage of unmetabolized ^{11}C-metoclopramide in plasma at different time points.



SUPPLEMENTAL FIGURE 1. Individual $k_{E,brain}$ values in whole brain grey matter (A) and $k_{E,plasma}$ values (B) for scans without (baseline) and with CsA infusion. Shown *P* values are from two sided, paired t-test. Correlation of percentage change in $k_{E,brain}$ and $k_{E,plasma}$ in response to CsA. (C) (*r* = Pearson correlation coefficient).



SUPPLEMENTAL FIGURE 2. Time-activity curves in whole brain grey matter for scan without (baseline) and with CsA infusion and fits obtained with the 1T2K model for one representative subject.



SUPPLEMENTAL FIGURE 3. Mean parametric K_1, k_2 and V_T images (n = 10) in Talairach space for baseline scans without CsA infusion. The basal ganglia, a dopamine D_2 receptor rich structure containing the ROIs caudate nucleus and putamen, are marked with a red broken line.

SUPPLEMENTAL TABLE 1

Descriptive Pharmacokinetic Parameters for Different Brain Regions for Scans without (Baseline) and with CsA Infusion

Brain region	Condition	C_{max} (SUV)	T_{max} (min)	$C_{55\ min}$ (SUV)	AUC (SUV.min)	$k_{E,brain}$ (1/min)
Whole brain grey matter	Baseline	1.06 ±0.19	7.0 ± 1.2	0.67 ± 0.10	47 ± 8	0.009 ± 0.001
	CsA	0.97 ± 0.26	10.5 ± 3.2*	0.75 ± 0.16*	47 ± 12	0.006 ± 0.002*
White matter	Baseline	0.61 ± 0.16	14.8 ± 6.4	0.50 ± 0.19	33 ± 5	0.005 ± 0.004
	CsA	0.62 ± 0.15	26.8 ± 12.3*	0.59 ± 0.12	32 ± 8	0.001 ± 0.002*
Frontal lobe mid gyrus	Baseline	1.07 ± 0.19	6.7 ± 1.6	0.67 ± 0.11	48 ± 8	0.009 ± 0.001
	CsA	0.99 ± 0.26	13.3 ± 5.3*	0.76 ± 0.16*	48 ± 11	0.006 ± 0.002*
Putamen	Baseline	1.21 ± 0.24	9.5 ± 2.5	0.83 ± 0.18	60 ± 11	0.010 ± 0.002
	CsA	1.15 ± 0.33	23.3 ± 18.5*	0.94 ± 0.24	57 ± 16	0.006 ± 0.003*
Caudate nucleus	Baseline	0.97 ± 0.17	11.6 ± 3.1	0.70 ± 0.16	46 ± 9	0.007 ± 0.001
	CsA	0.90 ± 0.19	21.3 ± 14.6	0.78 ± 0.17	45 ± 10	0.004 ± 0.002*
Occipital lobe medial part	Baseline	1.08 ± 0.18	6.3 ± 1.6	0.69 ± 0.10	49 ± 8	0.009 ± 0.001
	CsA	1.02 ± 0.27	9.3 ± 3.5*	0.78 ± 0.16*	49 ± 11	0.006 ± 0.002*
Cerebellum	Baseline	1.15 ± 0.21	4.4 ± 1.5	0.61 ± 0.09	47 ± 8	0.011 ± 0.001
	CsA	1.04 ± 0.30	6.5 ± 2.5*	0.69 ± 0.15*	47 ± 12	0.008 ± 0.002*
Pituitary gland	Baseline	4.06 ± 0.64	2.0 ± 0.9	2.20 ± 0.63	160 ± 270	0.009 ± 0.007
	CsA	3.61 ± 0.50	2.5 ± 1.5	2.33 ± 0.57	149 ± 28	0.007 ± 0.010

Values are reported as mean ± standard deviation

C_{max}, maximum concentration; T_{max}, time of C_{max}; AUC, area under the time-activity curve; $k_{E,brain}$, elimination slope for radioactivity washout from the brain

* $P < 0.05$ for comparison of CsA scan with baseline scan using a two sided, paired t-test

SUPPLEMENTAL TABLE 2

Outcome Parameters of the Reversible 1T2K Model in Different Brain Regions for Scans without (Baseline) and with CsA Infusion

Brain region	Condition	K_1 (mL/(cm^3.min))	k_2 (1/min)	V_T (mL/cm^3)	V_b (mL)	V_T / f_P
Whole brain	Baseline	0.092±0.014 (2±1)	0.044±0.005 (4±1)	2.1±0.3 (3±1)	0.043±0.008 (4±1)	10.4±2.7
grey matter	CsA	0.099±0.012 (2±0)	0.037±0.004 (4±1)*	2.7±0.4 (3±1)*	0.042±0.006 (4±1)	11.1±3.8
White matter	Baseline	0.027±0.004 (11±2)†	0.092±0.015 (21±3)†	0.3±0.0 (15±2)†	0.026±0.004 (8±1)	1.5±0.5†
	CsA	0.051±0.005 (3±1)*	0.022±0.003 (8±3)*‡	2.4±0.4 (6±2)*	0.023±0.003 (6±2)*	9.7±3.4*
Frontal lobe mid gyrus	Baseline	0.087±0.010 (2±1)	0.043±0.005 (4±3)	2.0±0.3 (3±2)	0.032±0.004 (5±3)	10.1±3.1
	CsA	0.097±0.009 (3±1)*	0.036±0.005 (5±3)*	2.8±0.5 (3±2)*	0.032±0.004 (6±4)	11.3±3.3*
Putamen	Baseline	0.091±0.015 (2±1)	0.035±0.003 (5±2)	2.6±0.4 (4±2)	0.037±0.007 (3±1)	12.9±3.2
	CsA	0.106 ± 0.019 (3±1)*	0.029±0.005 (5±3)*	3.7±0.7 (5±3)*	0.031±0.005 (3±1)*	14.9±4.2*
Caudate nucleus	Baseline	0.069±0.009 (2±1)	0.032±0.005 (6±3)†	2.2±0.5 (4±2)	0.029±0.007 (3±1)	10.9±3.3
	CsA	0.079 ± 0.011 (2±1)*	0.026± 0.005 (5±2)*	3.1±0.5 (4±2)*	0.025±0.005 (3±1)	12.6±3.5*
Occipital lobe medial part	Baseline	0.089±0.009 (2±0)	0.043±0.004 (4±2)	2.1±0.3 (4±1)	0.052±0.007 (4±1)	10.4±3.3
	CsA	0.104±0.009 (3±1)*	0.037±0.005 (6±3)*	2.8±0.5 (4±2)*	0.040±0.012 (3±1)	11.6±4.1*
Cerebellum	Baseline	0.098±0.010 (2±1)	0.053±0.006 (4±1)	1.9±0.3 (3±1)	0.045±0.011 (4±1)	9.4±3.0
	CsA	0.116±0.016* (3±1)	0.047±0.008 (5±2)*	2.5±0.4 (4±2%)*	0.042±0.006 (5±1)*	10.3±3.7
Pituitary gland	Baseline	0.384±0.055 (5±2)†	0.056±0.014 (12±3)†	7.3±2.0 (9±2)†	0.144±0.056 (13±7)	37.3±15.2†
	CsA	0.454±0.126 (7±3)‡	0.056±0.023 (15±6)‡	9.0±3.0 (11±5)‡	0.148±0.103 (12±6)	36.9±15.6‡

Values are reported as mean ± standard deviation. The value in parentheses represents the precision of parameter estimates (expressed as their coefficient of variation in percent).

K_1 (mL/(cm^3.min)), rate constant for radioactivity transfer from plasma into brain; k_2 (1/min), rate constant for radioactivity transfer from brain into plasma; V_T (mL/cm^3), total volume of distribution; V_T / f_P, V_T over the free fraction of ^{11}C-metoclopramide in plasma; V_b, fractional arterial blood volume in the brain

* P<0.05 for comparison of CsA scan with baseline scan using a two sided, paired t-test

† $P<0.05$ for comparison with whole brain grey matter region in baseline scans using one-way ANOVA followed by a Tukey's multiple comparison test
‡ $P<0.05$ for comparison with whole brain grey matter region in CsA scans using one-way ANOVA followed by a Tukey's multiple comparison test

SUPPLEMENTAL REFERENCES

1. Hamwi A, Salomon A, Steinbrugger R, Fritzer-Szekeres M, Jager W, Szekeres T. Cyclosporine metabolism in patients after kidney, bone marrow, heart-lung, and liver transplantation in the early and late posttransplant periods. *Am J Clin Pathol.* 2000;114:536-543.