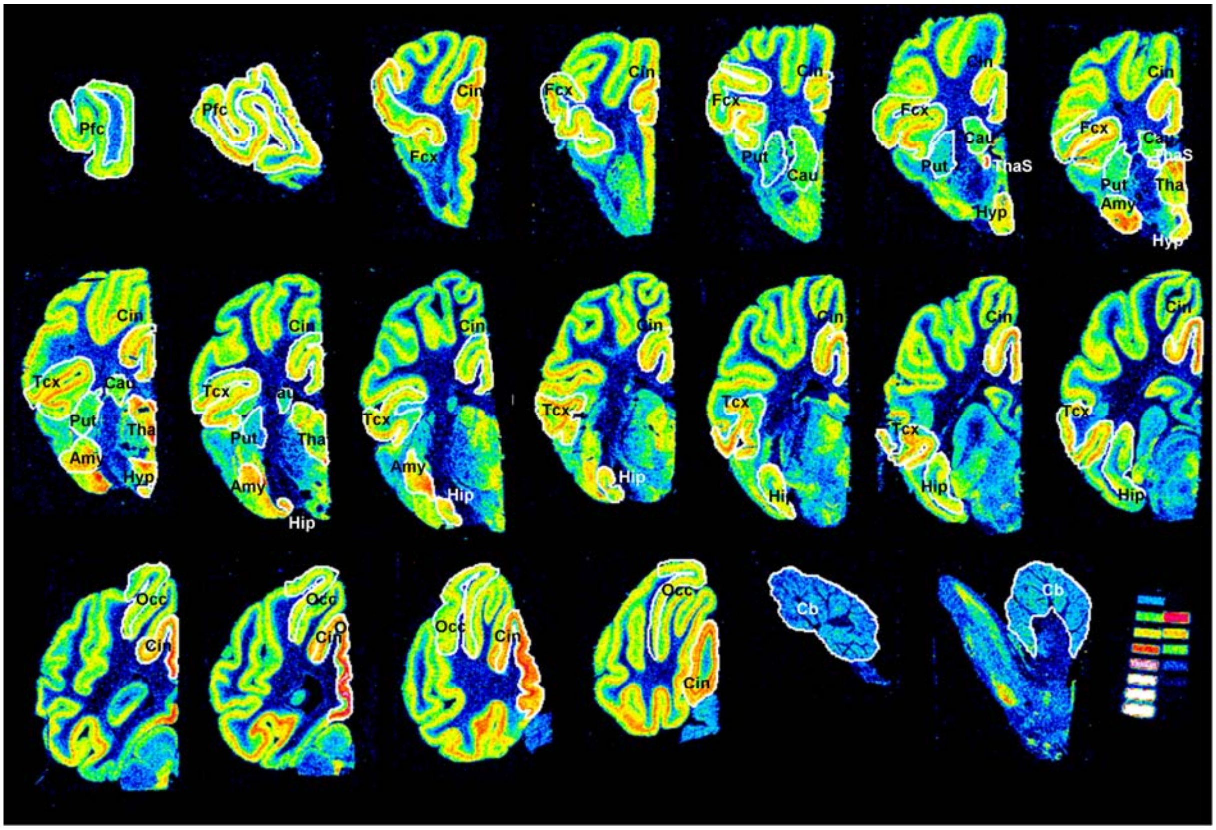
## SUPPLEMENTAL INFORMATION

### General methods

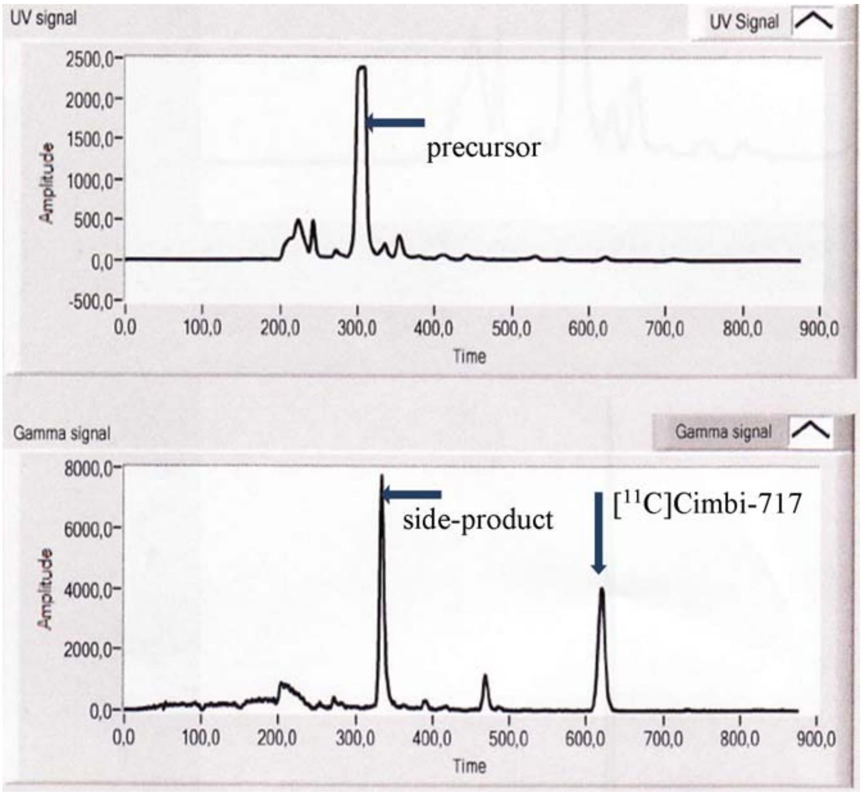
Chemicals were purchased from Acros, Fluka, Sigma, Tocris, or Merck. Unless otherwise stated, all chemicals were used without further purification. Thin-layer chromatography (TLC) was performed using plates from Merck (silica gel 60 $F_{254}$ and aluminium oxide 60 $F_{254}$). Analytical high-performance liquid chromatography (HPLC) measurements were performed on a Dionex system consisting of a P680A pump, a UVD 170U detector, and a Scansys radiodetector. Chemical purity was checked either by HPLC or by GC. [$^{11}$C]Methane was produced via the $^{14}N(p,\alpha)^{11}C$ reaction by bombardment of an [$^{14}$N]N2 containing 10% $H_2$ target with a 17 MeV proton beam in a Scanditronix MC32NI cyclotron. Radioactive syntheses were carried out on an automated Scansys module.

### Animal procedure

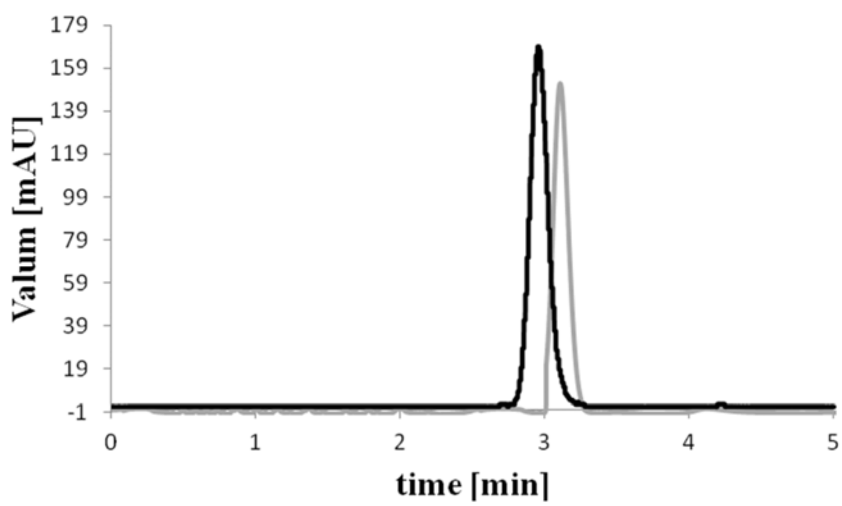
After arrival, animals were housed under standard conditions and were allowed to acclimatize for one week before scanning. To minimize stress, the animals were provided with straw bedding and environment enrichment, in the form of plastic balls and metal chains. On the scanning day, pigs were tranquilized by intramuscular (i.m.) injection of 0.5 mg/kg midazolam. Anesthesia was induced by i.m. injection of a Zoletil veterinary mixture (1.25 mg/kg tiletamin, 1.25 mg/kg zolazepam, and 0.5 mg/kg midazolam [Virbac Animal Health]). Following induction, anesthesia was maintained by intravenous (i.v.) infusion of 15 mg/kg/h propofol (B. Braun Melsugen AG). During anesthesia, animals were endotracheally intubated and ventilated (volume, 250 mL; frequency, 16 per min). Venous access was granted through 2 catheters (Becton Dickinson) in the peripheral milk veins, and an arterial line for blood sampling measurement was obtained by a catheter in the femoral artery after a minor incision. Vital signs including blood pressure, heart rate, blood oxygen saturation, and temperature were monitored throughout the duration of the PET scanning. Immediately after scanning, animals were sacrificed by i.v. injection of pentobarbital/lidocaine.




**Supplemental Fig. 1**: Autoradiography with 5 nM $^{3}$H-SB-269970. One hemisphere of a pig brain was sliced into 20 different regions. All regions are cut coronal except the 2 cerebellar regions, which are sliced in the sagittal plane. ROIs are indicated on all regions. Radioactivity of the steps of the left microscale used as internal standard are 28, 40, 68, 101, 167, 248, 397, and 630 fmol/mg TE (top to bottom).




**Supplemental Fig. 2:** Semipreparative HPLC chromatograms of $^{11}$C-Cimbi-717.




**Supplemental Fig. 3:** Analytic HPLC chromatograms of Cimbi-717 (black) and $^{11}$C-Cimbi-717 (gray).

**Supplemental Table 1:** Tested Radiolabeling Conditions for the Synthesis of $^{11}$C-Cimbi-717

| Precursor (mg) | Synthon | Base | Temperature (°C) | Solvent | Time (min) | RCY (%) |
|---|---|---|---|---|---|---|
| 0.3 | $^{11}$C-MeI | 1 equiv. NaOH | 60 | MeCN | 5 | 1 |
| 0.3 | $^{11}$C-MeI | 1 equiv. $K_2CO_3$ | 60 | MeCN | 5 | 2 |
| 0.3 | $^{11}$C-MeI | 10 equiv. NaOH | 60 | MeCN | 5 | 8 |
| 0.3 | $^{11}$C-MeOTf | 1 equiv. $K_2CO_3$ | 60 | MeCN | 5 | 2 |
| 0.3 | $^{11}$C-MeOTf | 10 equiv. $K_2CO_3$ | 60 | MeCN | 5 | 27 |
| 0.3 | $^{11}$C-MeOTf | 10 equiv. $K_2CO_3$ | 60 | Acetone | 5 | 18 |
| 0.3 | $^{11}$C-MeOTf | 10 equiv. $Bu_4NOH$ | 60 | MeCN | 5 | - |
| 0.3 | $^{11}$C-MeOTf | 10 equiv. $Cs_2CO_3$ | 60 | MeCN | 5 | 15 |
| 0.3 | $^{11}$C-MeOTf | No base | 60 | MeCN | 5 | 2 |
| 0.3 | $^{11}$C-MeOTf | 20 equiv. NaOH | 60 | MeCN | 5 | 14 |
| 0.3 | $^{11}$C-MeOTf | 20 equiv. $K_2CO_3$ | 60 | MeCN | 5 | 22 |
| 0.5 | $^{11}$C-MeOTf | 10 equiv. $K_2CO_3$ | 60 | MeCN | 5 | 23 |
| 0.3 | $^{11}$C-MeOTf | 10 equiv. $K_2CO_3$ | 60 | DMSO | 5 | 12 |
| 0.3 | $^{11}$C-MeOTf | 10 equiv. $K_2CO_3$ | 60 | DMF | 5 | 11 |
| 0.3 | $^{11}$C-MeOTf | 10 equiv. $K_2CO_3$ | 80 | MeCN | 5 | 23 |
| 0.3 | $^{11}$C-MeI | 10 equiv. $K_2CO_3$ | 120 | DMSO | 5 | 4 |

**Supplemental Table 2**: Regional Modeling Results for $^{11}$C-Cimbi-717 PET Scanning in the Pig Brain

| | 1-tissue compartment model | | | | 2-tissue compartment model | | | | Logan linerization model | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Baseline | | SB-269970 | | Baseline | | SB-269970 | | Baseline | | SB-269970 | |
| Region | $V_T$ | AIC | $V_T$ | AIC | $V_T$ | AIC | $V_T$ | AIC | $V_T$ | Start lin. | $V_T$ | Start lin. |
| L Cortex | 11.6 ± 1.90 | 5.45 ± 40.8 | 6.25 ± 0.63 | -1.83 ± 9.40 | 11.7 ± 1.58 | -3.12 ± 41.2 | 6.33 ± 0.62 | 5.30 ± 14.1 | 11.7 ± 1.57 | 17 ± 5 | 6.44 ± 0.63 | 20 ± 14 |
| R Cortex | 11.9 ± 2.16 | 2.17 ± 43.7 | 6.30 ± 0.76 | 4.70 ± 16.3 | 12.1 ± 2.04 | -0.75 ± 36.1 | 6.58 ± 0.69 | 11.4 ± 22.5 | 12.3 ± 2.24 | 15 ± 8 | 6.52 ± 0.82 | 18 ± 12 |
| R cerebellum | 7.22 ± 1.41 | 43.2 ± 38.8 | 3.96 0.44 | 36.7 ± 14.4 | 7.18 ± 1.32 | 35.6 ± 30.5 | 4.04 ± 0.47 | 42.4 ± 12.3 | 6.50 ± 1.24 | 14 ± 7 | 3.68 ± 0.34 | 8 ± 3 |
| L cerebellum | 6.40 ± 1.02 | 49.1 ± 26.3 | 3.76 ± 0.38 | 35.7 ± 14.7 | 6.55 ± 0.88 | 44.0 ± 29.2 | 3.97 ± 0.62 | 43.2 ± 12.3 | 5.94 ± 1.03 | 14 ± 6 | 3.50 ± 0.28 | 7 ± 3 |
| L frontal white matter | 12.8 ± 2.19 | 7.00 ± 28.7 | 6.78 ± 0.68 | 6.81 ± 22.3 | 13.4 ± 2.02 | -1.02 ± 32.8 | 6.85 ± 0.70 | 6.90 ± 19.9 | 13.3 ± 2.34 | 19 ± 6 | 6.94 ± 0.75 | 20 ± 9 |
| R frontal white matter | 13.0 ± 2.36 | -0.63 ± 41.6 | 6.78 ± 0.74 | 7.71 ± 16.9 | 13.4 ± 2.18 | -4.91 ± 39.7 | 6.94 ± 0.87 | 10.5 ± 9.79 | 14.2 ± 2.80 | 21 ± 5 | 7.13 ± 0.80 | 25 ± 0 |
| R hippocampus | 13.6 ± 2.10 | 74.9 ± 21.9 | 6.61 ± 0.37 | 74.8 ± 28.1 | 12.4 ± 1.79 | 73.0 ± 17.5 | 6.79 ± 0.42 | 77.7 ± 30.1 | 11.5 ± 3.13 | 48 ± 8 | 6.25 ± 1.32 | 39 ± 23 |
| L hippocampus | 12.9 ± 2.49 | 68.9 ± 20.8 | 6.19 ± 0.31 | 76.3 ± 20.0 | 13.3 ± 2.54 | 72.1 ± 19.7 | 6.30 ± 0.32 | 78.9 ± 21.1 | 11.6 ± 4.23 | 42 ± 8 | 5.75 ± 1.33 | 28 ± 12 |
| L lateral thalamus | 15.3 ± 2.03 | 57.1 ± 29.5 | 7.49 ± 0.25 | 81.7 ± 24.6 | 15.4 ± 2.00 | 63.8 ± 29.4 | 7.56 ± 0.25 | 88.9 ± 23.7 | 13.7 ± 1.68 | 38 ± 14 | 7.20 ± 0.76 | 43 ± 16 |
| R lateral thalamus | 16.1 ± 2.89 | 53.9 ± 23.0 | 7.61 ± 0.64 | 82.4 ± 17.1 | 16.4 ± 2.84 | 57.3 ± 25.1 | 8.04 ± 1.20 | 90.0 ± 22.2 | 15.1 ± 3.14 | 36 ± 14 | 7.83 ± 1.37 | 25 ± 11 |
| R medial thalamus | 17.3 ± 2.93 | 69.2 ± 24.1 | 7.17 ± 0.38 | 90.4 ± 22.3 | 18.1 ± 3.08 | 66.8 ± 19.8 | 7.41 ± 0.45 | 92.5 ± 21.2 | 14.7 ± 2.39 | 50 ± 12 | 6.80 ± 0.63 | 30 ± 10 |
| L medial thalamus | 16.5 ± 2.41 | 59.3 ± 28.6 | 6.97 ± 0.56 | 74.6 ± 35.0 | 17.2 ± 2.60 | 55.8 ± 30.3 | 7.11 ± 0.57 | 78.6 ± 33.7 | 14.3 ± 3.11 | 43 ± 8 | 6.68 ± 0.69 | 30 ± 16 |
| L putamen | 14.1 ± 2.79 | 71.2 ± 25.5 | 7.29 ± 0.42 | 102 ± 22.8 | 14.3 ± 2.53 | 60.1 ± 12.3 | 7.44 ± 0.31 | 108 ± 22.6 | 12.8 ± 1.53 | 39 ± 12 | 7.16 ± 1.02 | 35 ± 22 |
| R putamen | 13.9 ± 3.35 | 78.2 ± 21.7 | 7.18 ± 0.81 | 88.6 ± 10.6 | 14.1 ± 3.35 | 76.0 ± 15.3 | 7.38 ± 0.64 | 92.0 ± 14.0 | 13.4 ± 2.97 | 32 ± 13 | 7.48 ± 0.52 | 28 ± 13 |
| R caudate | 13.1 ± 2.00 | 63.0 ± 18.3 | 6.73 ± 0.51 | 86.7 ± 34.9 | 13.6 ± 1.95 | 59.6 ± 13.3 | 7.19 ± 0.04 | 71.9 ± 5.35 | 11.8 ± 1.31 | 40 ± 18 | 7.17 ± 0.28 | 37 ± 16 |
| L caudate | 12.7 ± 1.58 | 70.8 ± 14.4 | 6.94 ± 0.42 | 82.4 ± 25.4 | 12.3 ± 0.83 | 71.3 ± 12.8 | 7.03 ± 0.45 | 83.7 ± 28.8 | 12.2 ± 2.75 | 44 ± 18 | 6.94 ± 1.06 | 32 ± 12 |
| Converged regions | 94/96 | | 79/80 | | 85/96 | | 76/80 | | 90/96 | | 79/80 | |

Kinetic modelling outcome at baseline (n=6) and following SB-269970 pre-treatment (n=5, various doses) is shown. Mean values and S.D. are given.
$V_T$: Distribution volume. AIC: Akaike Information Criteria.