Supplemental materials and methods

Tracer production

[^{11}C]-(*R*)-PK11195 was synthesised according to methods first described by (*1*) and (*2*), with some modifications. Briefly, [^{11}C]CH_3I was produced from [^{11}C]CO_2 or [^{11}C]CH_4 generated by irradiating N_2 with protons in the presence of 0.2% O_2 or 5% H_2. [^{11}C]CH_3I was then reacted with desmethyl-(*R*)-PK11195 (Tocris Cookson) forming [^{11}C]-(*R*)-PK11195. [^{18}F]GE-180 was synthesised using the previously published method (*3*). Briefly, [^{18}F]F^- was generated by proton irradiation of ^{18}O-enriched H_2O (97% enrichment). After suitable workup the [^{18}F]F-anion was then reacted with the precursor molecule (GE Healthcare) resulting in the formation of [^{18}F]GE-180. Both tracers were subsequently purified with semi-preparative HPLC and formulated for injection.

Intrastriatal injection

The rats were placed onto a stereotaxic frame (Kopf Instruments) and the skull was exposed. The brain of the animal was then exposed *via* a hole, which was drilled though the skull. A pulled glass cannula was inserted into the left striatum using the following stereotaxic coordinates from bregma: + 1 mm anterior/posterior, − 3 mm medial/lateral) − 4 mm dorsal/ventral. Lipopolysaccharide (LPS) (*E. coli* 026:B6, Sigma-Aldrich; 10 μg/μl or 1 μg/μl) in phosphate buffered saline (PBS, 1 μL, 0.1 M) was then injected over 5 min (0.2 μL/min) followed by a 2 minute rest period to allow the solution to diffuse into the brain prior to the removal of the capillary (*4*). In total 18 rats were injected with LPS. A saline control group was also included in the study (n = 12). Here, the same surgery, as described above, was performed, but only PBS (0.5 μL to 2 μL) was injected intracerebrally.

In vivo imaging

In vivo imaging was performed using an Inveon multimodality PET/ CT (Siemens Medical Solutions) small animal scanner with approximately 1.3 mm resolution. The device generates images with 159 transaxial slices with a field of view of 10 cm. A CT scan was performed in order to correct for signal

attenuation in the PET scan and provide anatomical references. Two different dynamic emission scans (duration 60 min for ^{11}C and 90 min for ^{18}F) were acquired in list mode for these studies. The dynamic scans were started immediately after the intravenous injection of the required radiotracer (Supplemental Table 1).

In vivo data analysis

The PET data was reconstructed using the OSEM iterative reconstruction protocols in the Inveon acquisition software (Siemens Medical Solutions). The images were evaluated by drawing a spherical volume of interest (VOI) in the injected striatum. This VOI was then mirrored in the contralateral hemisphere to act as a reference region. VOI analysis and segmentation was performed in Carimas v2.6 (University of Turku). The time activity curve (TAC) from the VOI drawn in the contralateral hemisphere was subtracted from the TAC from the lesioned hemisphere (Supplemental Fig. 2) to examine the point of maximal binding (25-50 min) during the imaging timeframe. The binding potential (bound to free ratio) was calculated over the period of maximal binding, as per the literature procedure set out by (*5*). Briefly, the area under the curve was calculated by integrating between the defined time limits during the maximal binding period for both the lesioned TAC and the contralateral TAC. In order to get the binding potential the following calculation was performed:

$$BP_{in\ vivo} = (TAC_{(Lesion)} - TAC_{(Contralateral)})/TAC_{(Contralateral)}$$

Immunohistochemical analysis

Fresh tissue from animals in set A was post-fixed for 20 min using the fixative periodate-lysine-paraformaldehyde containing 0.1% glutaraldehyde (*6*). The endogenous peroxidase activity was quenched by placing the sections in a solution of H_2O_2 (3%, Sigma Aldrich) in MeOH (Sigma Aldrich). The slides were then blocked using normal horse serum (10% in PBS) for 1 h, washed with PBS, and incubated with the requisite primary antibody (mouse-anti-OX-42 for microglia, Serotec; or goat-anti-GFAP for activated astrocytes, Millipore). The slides were washed prior to the addition of the

required biotinylated secondary antibody (rat adsorbed horse-anti-mouse IgG for OX-42 or horse-anti-goat IgG for GFAP; Vector). The immunostaining was then developed using DAB and slides were counter stained with cresyl violet.

Supplemental results

Radiochemistry

PK11195 and GE-180 were successfully labelled with the short-lived radioactive isotopes, ^{11}C and ^{18}F respectively and this was confirmed by radio-HPLC (Supplemental Figure 1). The tracers were produced with good radiochemical purity (values measured at end of synthesis), >99% for ^{11}C-(*R*)-PK11195 with a specific radioactivity of 51±11 MBq/nmol starting from ^{11}C-CO_2 (n=3) or 680±310 MBq/nmol starting from ^{11}C-CH_4 (n=6). The corresponding values for ^{18}F-GE-180 were 96.0±0.8% and >1000 MBq/nmol (n=6). The radiochemical purity remained unchanged for both tracers during the time course of their use. The CH4 production methods of ^{11}C-(R)-PK11195 resulted in an increase in specific activities compared to the CO2 method. However, the images and binding potentials were unaffected by the method of production as the specific activity of the product was sufficiently high to microdose.

Point of maximal binging

From the TACs calculated by subtracting the contralateral TAC from the lesion TAC (Supplemental Fig. 2), the maximal binding during the dynamic PET scans was between 25-50 minutes.

References

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3. Wadsworth H, Jones PA, Chau W-F, et al. ^{18}F-GE-180: A novel fluorine-18 labelled PET tracer for imaging Translocator protein 18kDa (TSPO). *Bioorg Med Chem Lett.* 2012;22:1308-1313.

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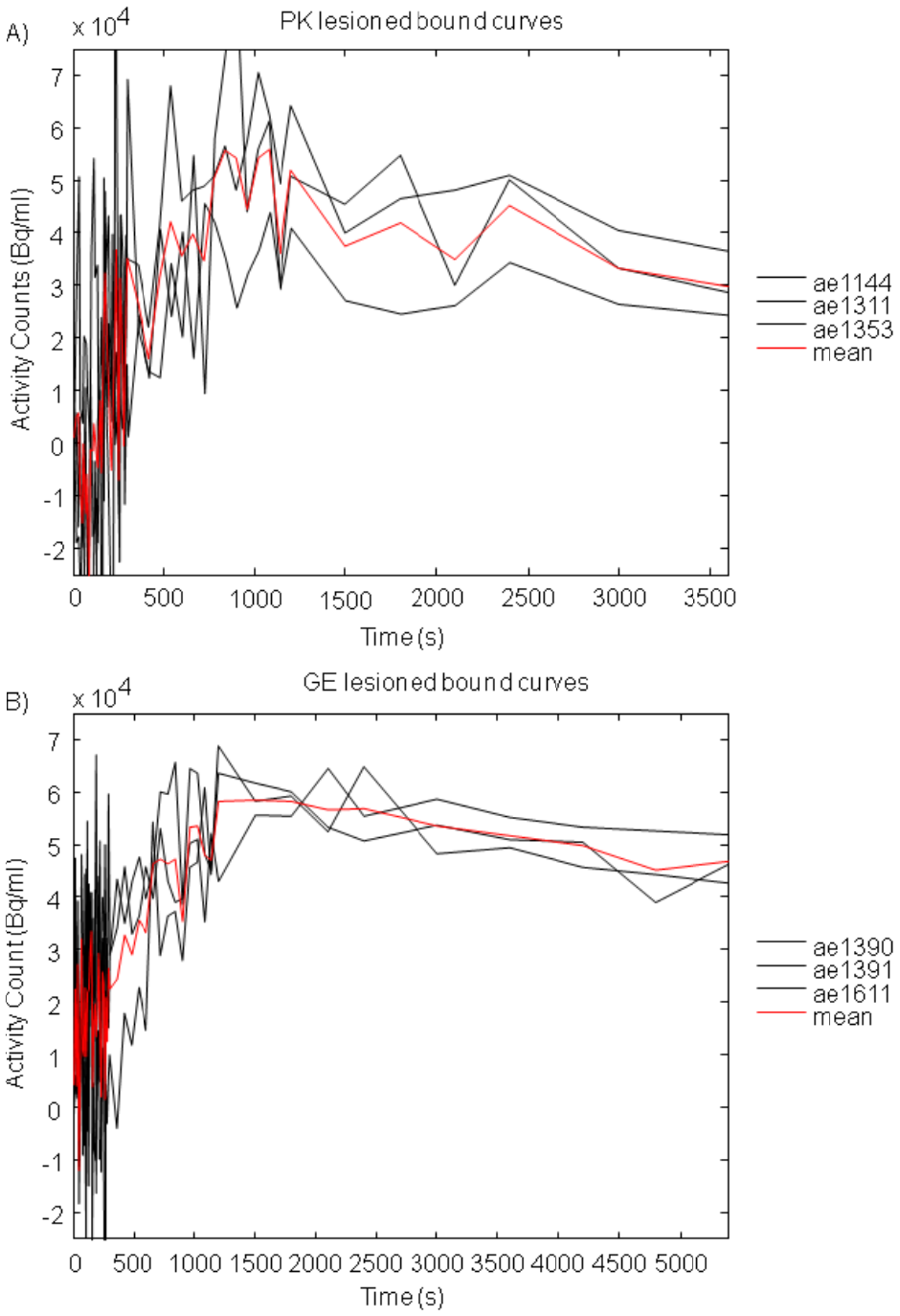
A)

	[11C]PK11195			[18F]GE-180		
	Saline	1 μg LPS	10 μg LPS	Saline	1 μg LPS	10 μg LPS
Weight/g	385 ± 57	326 ± 10	324 ± 28	474 ± 18	347 ± 24	323 ± 45
Injected radioactivity MBq	47 ± 14	65 ± 28	54 ± 15	39 ± 8	48 ± 12	44 ± 4

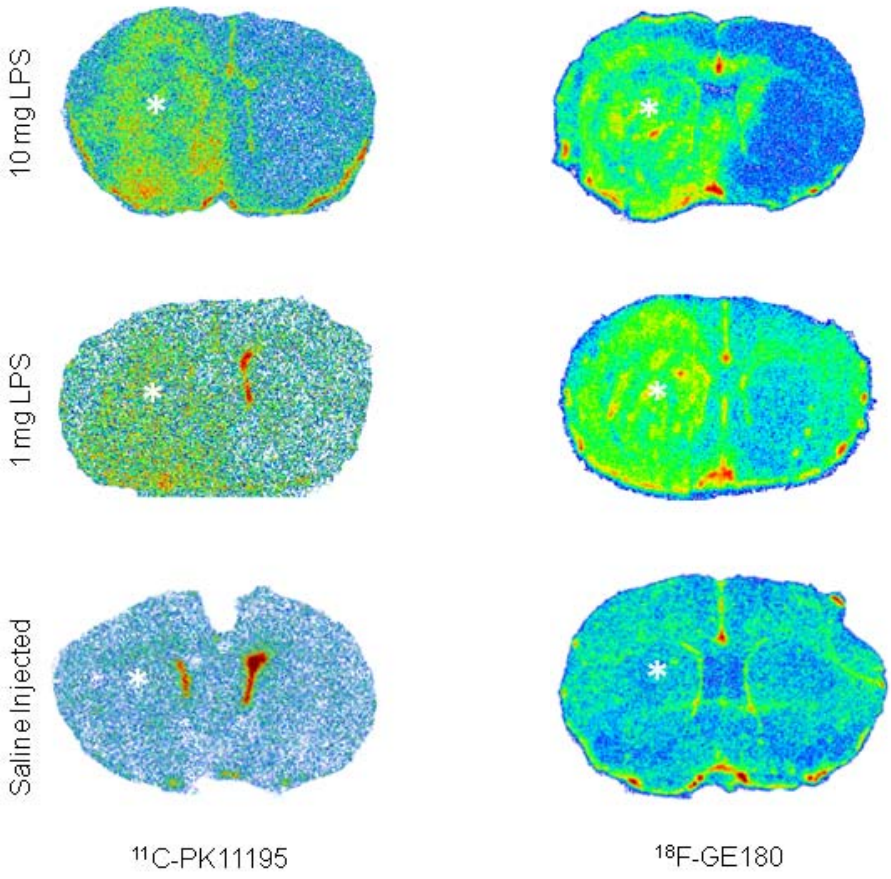
B)

	[11C]PK11195		[18F]GE-180	
	Saline	10 μg LPS	Saline	10 μg LPS
Weight/g	420 ± 68	245 ± 109	398 ± 47	349 ± 93
Injected radioactivity MBq	42 ± 3	33 ± 20	37 ± 3	40 ± 7

Supplemental Figure 1: Tables demonstrating the average weights and the average injected radioactivity for the animals in each group. A) Data for animals in group A which were used in the *ex-vivo* and *in-vitro* autoradiography analysis. B) Data for animals in group B which were used in the *in-vivo* analysis.



Supplemental Figure 2: A) Graph showing the bound to free TAC, which was obtained from subtracting the TAC from the contralateral hemisphere from the TAC from the lesioned hemisphere. imaged with ^{11}C-PK11195. Red line shows the mean binding potential from the animals. B) Graph showing the bound to free TAC, which was obtained from subtracting the TAC from the contralateral hemisphere from the TAC from the lesioned hemisphere imaged with ^{18}F-GE-180.



Supplemental Figure 3: Representative coronal striatal autoradiography images when animals were imaged with [^{11}C]PK11195 (left hand side) and [^{18}F]GE-180 (right hand side). * Marks the site of injection.