SUPPLEMENTAL MATERIALS AND METHODS

PET Data Acquisition

All of the PET experiments were performed with two individual dedicated small animal PET scanners (microPET Inveon, Siemens, Erlangen, Germany) with a spatial resolution of 1.4 mm and an axial field of view of 12.7 cm. The PET data acquisition was performed as previously described [1]. Briefly, all of the animals were catheterized in the tail vein. One anesthetized rat or two anesthetized mice were placed on dedicated animal beds (Medres, Cologne, Germany) that contained a warming feedback control unit (including a rectal thermal sensor) to maintain the animal's core body temperature at 37°C during the PET measurements. Stereotactic head holders were used to avoid head motion artifacts.

The scan was started 5 s before injection of the radiotracer. A 90-min dynamic PET scan was performed for all of the bolus injection experiments, followed by a 13-min transmission scan using a rotating ^{57}Co source, which enables attenuation correction. The PET list-mode data were binned in 34 time frames (6 x 10 s, 6 x 30 s, 6 x 60 s and 16 x 300 s) and reconstructed with a 2D-filtered back projection algorithm, matrix size of 256 x 256 and a zoom factor of 2 (mice) and a matrix size of 128 x 128 (rats) resulting in a voxel size of 0.19 x 0.19 x 0.80 mm^3 for mice and 0.78 x 0.78 x 0.80 mm^3 for rats.

The PET data analysis was performed using PMOD software 3.2 (PMOD, Zürich, Switzerland). The PET images were coregistered to a brain template for rats [2] and mice [3]. Corresponding brain atlas volumes of interest (VOIs) and time–activity curves (TACs) were obtained after applying the brain template VOIs to the coregistered PET images.

Reproducibility

For the test–retest experiments one additional PET scan at high SA was performed seven days before the MLCTA in four rats and four mice. The high SA ranged from 87 to 179 GBq/μmol for the measurements in rats and from 52 to 116 GBq/μmol for the measurements in mice, which corresponds to an injected mass of 0.08 to 0.21 μg/kg and 2.1 to 2.9 μg/kg, respectively. Reproducibility was calculated with one-way ANOVA using equation 1.

$$reproducibility = 100 - \frac{\left|BP_{scan2} - BP_{scan1}\right|}{0.5 \times \left(BP_{scan1} + BP_{scan2}\right)} \quad (1)$$

Reliability (RL)

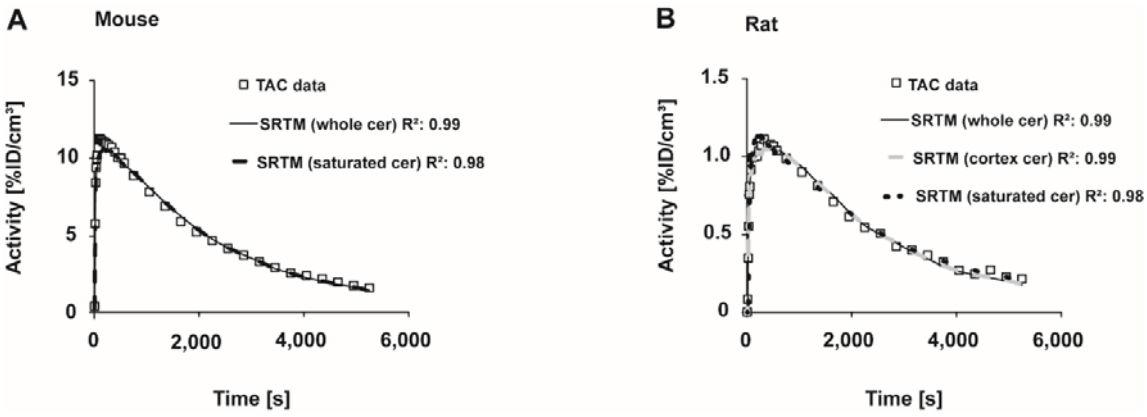
To evaluate within-subject variability relative to between-subject variability, the mean sum square both within the subjects (MSWS) and between the subjects (MSBS) was calculated using 1-way ANOVA and expressed as a percentage of the mean value:

$$RL = \frac{(MSBS - MSWS)}{(MSBS + MSWS)} \quad (2)$$

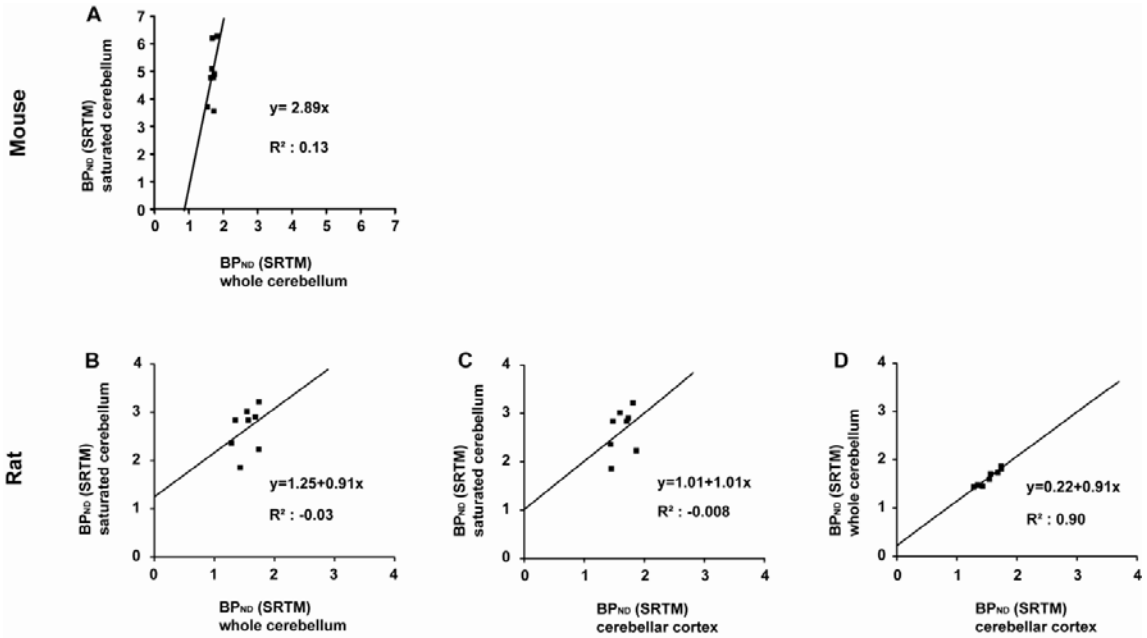
References

1. Fischer K, et al. Noninvasive nuclear imaging enables the in vivo quantification of striatal dopamine receptor expression and raclopride affinity in mice. *J Nucl Med.* 2011;52:1133–1141.
2. Schiffer WK, et al. Serial microPET measures of the metabolic reaction to a microdialysis probe implant. *J Neurosci Methods.* 2006;155:272–284.

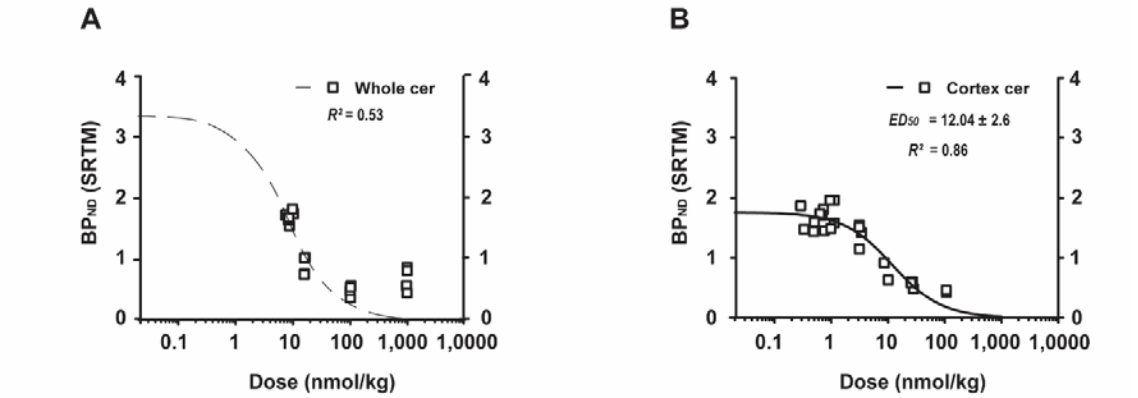
3. Mirrione MM, et al. A novel approach for imaging brain-behavior relationships in mice reveals unexpected metabolic patterns during seizures in the absence of tissue plasminogen activator. *Neuroimage.* 2007;38:34–42.



Supplemental Figure 1: Comparison of SRTM fits using different modifications of the reference region. Time–activity curves for the thalamus in one exemplary mouse (A) and rat (B) at high SA, analyzed using the SRTM with different modifications of the cerebellum as a reference region. The whole cerebellum and the saturated cerebellum were used as a reference region for the rats and mice. Additionally, the cerebellar cortex was applied as a reference tissue for the rat measurements. In both species, all reference regions revealed appropriate fits.



Supplemental Figure 2: Correlation of thalamic BP_{ND} in mice and rats using different reference region modifications. The BP_{ND} values at high SA were calculated using the SRTM with different modifications of the reference region in the rats and mice. The BP_{ND} values obtained using the saturated cerebellum as the reference region were plotted against the BP_{ND} values using the whole cerebellum as the reference region in both species (mouse: A; rat: B). Additionally, the cerebellar cortex was used in the rats (C). The correlation between BP_{ND} values obtained using the whole cerebellum and the cerebellar cortex as the reference region showed a coefficient of determination of 0.9 (D).



Supplemental Figure 3: Determination of the effective dose (ED_{50}) of [^{11}C]DASB in mice and rats. BP_{ND} values were plotted as a function of the injected dose in nmol/kg. The mouse data could not be fit using equation 4 (Supplemental Materials and Methods) (A). In contrast, the rat measurements revealed an ED_{50} of 12.0 ± 2.6 nmol/kg (B).

Supplemental Table 1: [^{11}C]DASB test–retest experiments in different brain regions**.** Mean BP_{ND} values of the thalamus, striatum and hippocampus of the mice and rats

Mouse (SRTM)	*BP_{ND} ± SD Scan I*	*BP_{ND} ± SD Scan II*	*Reproducibility* [%]	*Reliability* [%]
Thalamus	1.69 ± 0.04	1.70 ± 0.12	94.54	n.a.
Striatum	1.04 ± 0.05	1.07 ± 0.08	92.52	n.a.
Hippocampus	0.82 ± 0.03	0.81 ± 0.10	90.42	n.a.
Rat (SRTM)	*BP_{ND} ± SD Scan I*	*BP_{ND} ± SD Scan II*	*Reproducibility* [%]	*Reliability* [%]
Thalamus	1.68 ± 0.16	1.59 ± 0.19	92.00	43.43
Striatum	1.30 ± 0.15	1.28 ± 0.18	88.89	14.61
Hippocampus	1.15 ± 0.09	1.12 ± 0.13	90.94	3.97

Supplemental Table 2: Calculation of B_{avail} and $appK_D$ using different modifications of the reference region in rats.

Rat *Reference Tissue*	B_{avail} ± *SD* *[pmol/mL]*	$appK_D$ ± *SD* *[nM]*	R^2
Whole Cerebellum	3.5 ± 0.6	2.1 ± 0.4	0.86
Cerebellar Cortex	3.9 ± 0.7	2.2 ± 0.4	0.87
Saturated Cerebellum	2.9 ± 0.6	0.9 ± 0.2	0.89