Small Animal Imaging Benefits and Challenges

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Definitions: Molecular Imaging

- **The term molecular imaging can be broadly defined as the in vivo characterization and measurement of biologic processes at the cellular and molecular level. [Weissleder & Mahmood, Radiology 2001].**

- **MI techniques directly or indirectly monitor and record the spatiotemporal distribution of molecular or cellular processes for biochemical, biologic, diagnostic, or therapeutic applications [Thakur & Lentle, Radiology 2005].**

Imaging & "Molecular Targeting"

- **Interactions between a probe and a protein target using pre-genomic techniques.**
  - **"Biochemical probes" such as iodide (~50 years), receptor binding radiotracers and monoclonal antibodies (~25 years) from autopsy, linkage and drug efficacy, etc.**
- **Interactions between a probe and a protein target using post-genomic techniques.**
  - **Molecular biology, proteomics, genomics, antisense, reporter genes, protein-protein interactions. More targets (500 → 2000-3000)**

ATLAS scanner

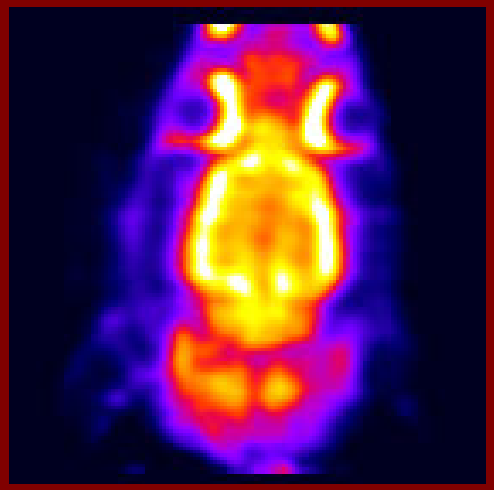
Advanced Technology Laboratory Animal Scanner

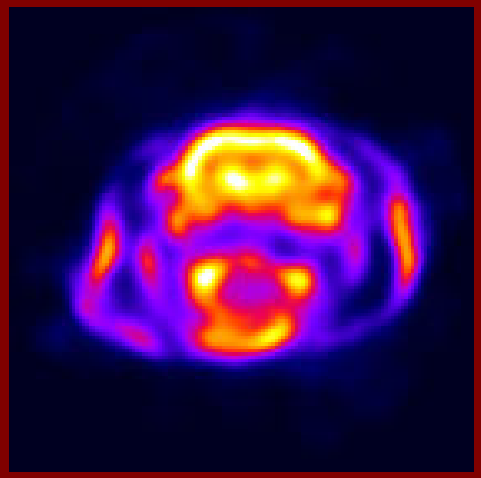
Technical Characteristics

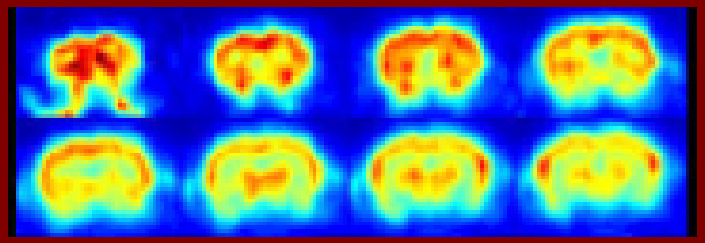
- 11.8 cm diameter aperture
- 2 cm axial field-of-view
- 18 phoswich detector modules consisting of 8 mm of LGSO and 7 mm of GSO (Hitachi, Japan)
- Reduced radial resolution loss with increasing radius
- Improved spatial sampling
- Other features:
  - dynamic imaging
  - whole-body imaging
  - routine 3D OSEM reconstruction

- High sensitivity 1.8% (250-700 keV)
  2.7% (100-700 keV)
- High Resolution 1.8 mm (FBP)
  1.5 mm (3D OSEM)

Rat Brain with [^{18}F]FDG







NIH ATLAS

eXplore Vista

Coronal **Transverse** **Transverse**

The Goal of Small Animal Imaging

- To be able to carry out experiments in rodents that are presently being carried out in humans including:
  - Pharmacokinetics
  - Repeat Studies in the same animal
  - Determination of biochemical parameters for radioligands
- To replace single-time-point dissection and autoradiography, which do not easily lead themselves to the above capabilities.

Benefits --- and --- Challenges

- **Decreased use of animals**
- **Statistical Advantage**
- **Complete kinetics**
- **Detailed biochemical parameters**
- **Screening of Drug Candidates**
- **Radioactive Dose Requirements**
- **Mass Requirements**
- **Blood Volume**
- **Anesthesia**
- **Bolus vs. Infusion**
- **Sensitivity for TAC vs. Images**

Why small animal PET scanning?

- The need to replace, reduce, and refine animal usage (Russell & Burch, 1959).
- The availability (and expense) of various animal models, mostly in mice.

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  - Paired Statistics
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Small animal imaging with ATLAS: can we reproduce human studies?

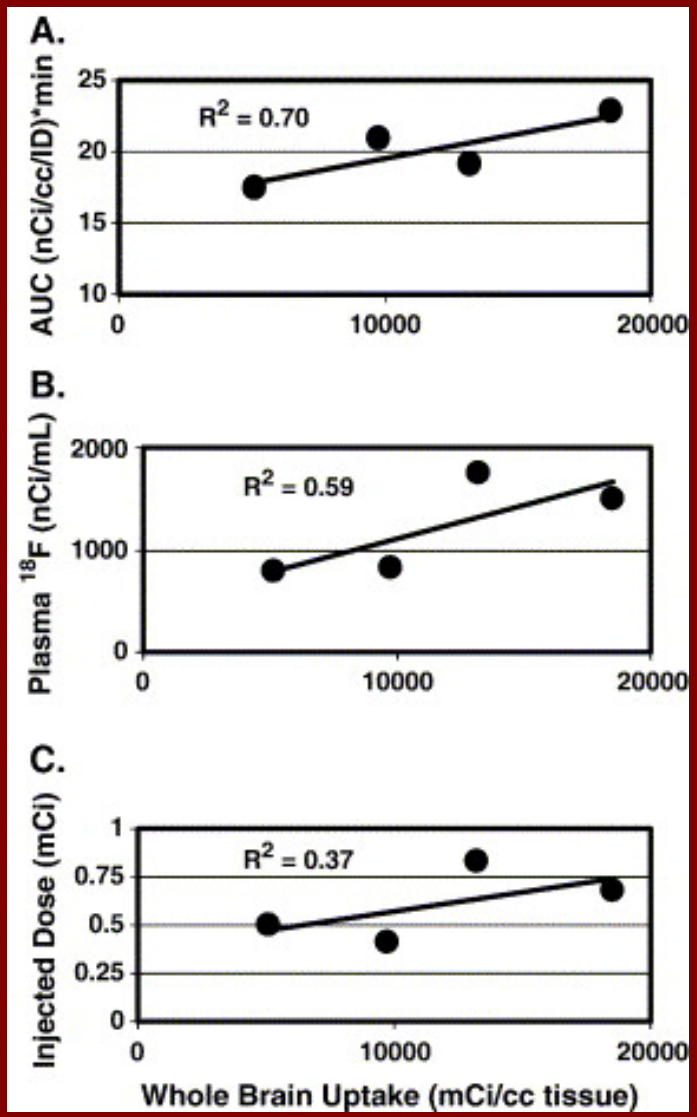
- PET scans of Target Tissue
  - With and without anesthesia
    - Sensitivity for anatomical definition (PET/CT)
    - Sensitivity for time activity curves.
- Input Function
    - Blood
    - Plasma
    - Metabolites
      - % parent
      - %ID parent
- Validation vs. Ex Vivo dissection or phosphorimaging.

Small animal imaging with ATLAS: can we reproduce human studies?

- **Methods for [^{18}F]FDG :**
  - **PET scans**
    - **following a bolus of ~48 MBq (1.3 mCi), [^{18}F]FDG were performed in rats with or without isoflurane.**
  - **Input Function**
    - **Arterial blood sampling was carried out throughout the uptake period. Plasma was counted.**
  - **Validation:**
    - **At 60 min the rat was killed, and the brain was rapidly removed and dissected into 5 structures (thalamus, cortex , brain stem, cerebellum, and half brain).**
    - **Activity in the tissue samples was compared to the mean activity of the last 5 min of calibrated PET data.**

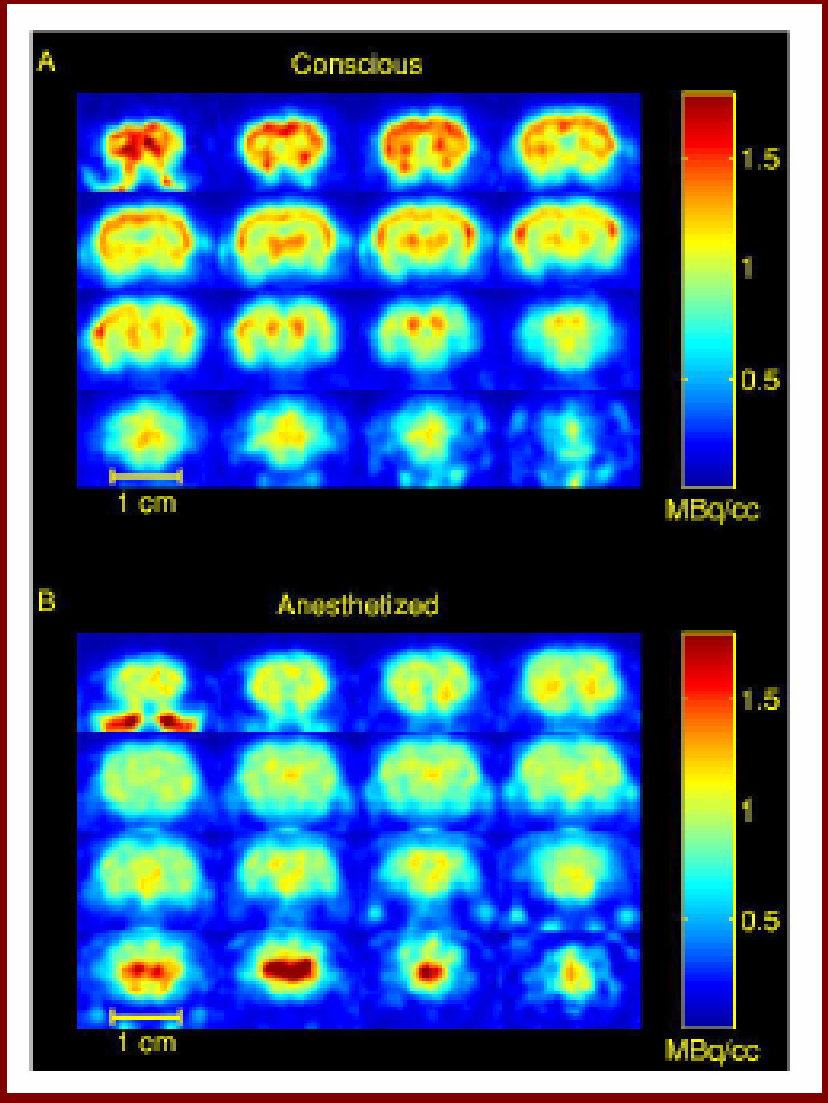
Alternative to IV injection, blood sampling and stress ?

- **Intraperitoneal injection**
  - **Normalize by various parameters = 15% CV**
  - **Normalize by whole brain radioactivity = 5%CV**
  - **AUC plasma correlated with whole brain uptake.**



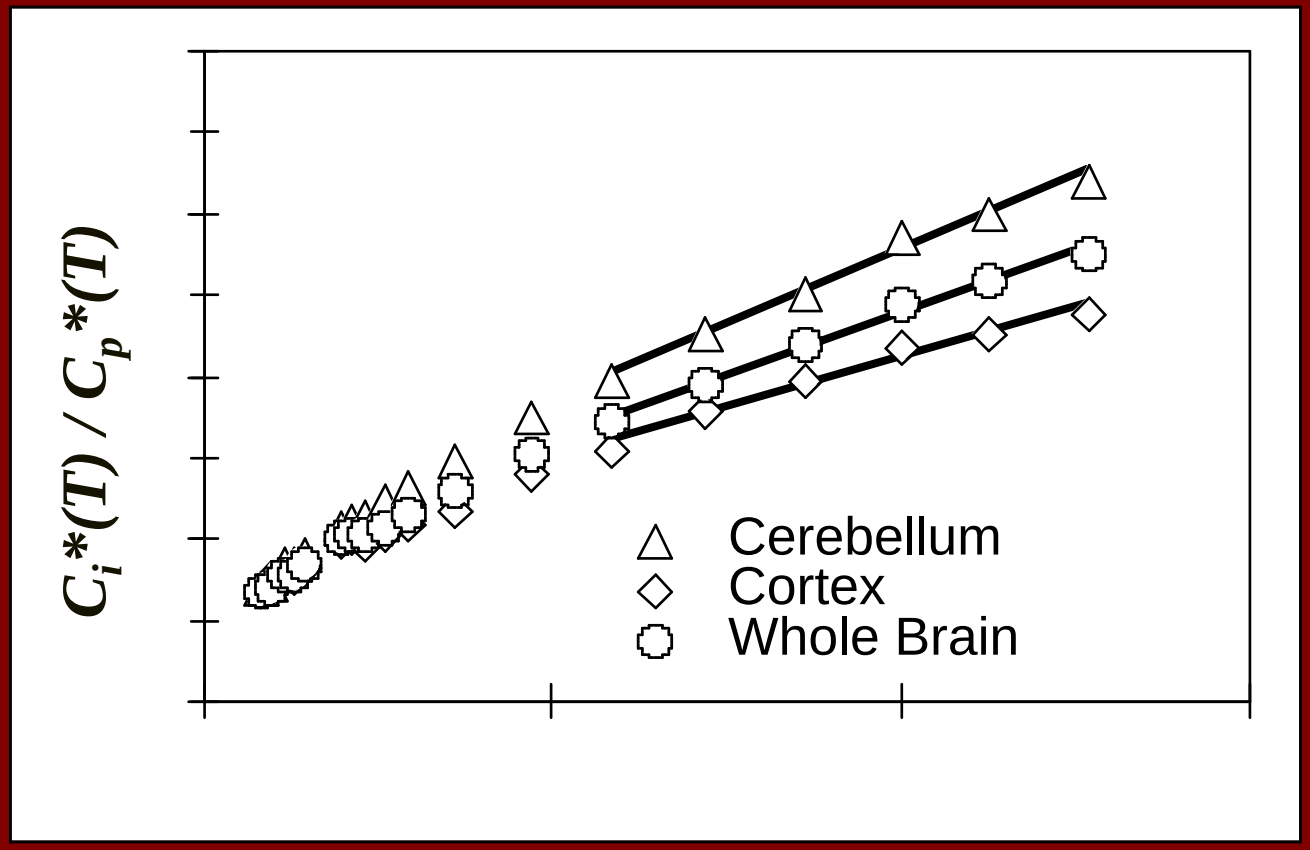
Marsteller et al. Nucl Med Biol. 2006

[^{18}F]FDG PET Image of the brain of a rat scanned for 60 min after a 45 min uptake period.



Taken from Shimoji et al. J Nucl Med 2004.

[^{18}F]FDG uptake in an anesthesized rat over 60 min.



Patlak plot of the TAC for the cerebellum, cortex, and whole brain. On the abscissa is the normalized plasma activity, $\theta(T)=Integral\ C_p^*(t)\ dt\ /C_p^*(T)$, where C_p^* is the arterial plasma concentration of [^{18}F]FDG. Tissue activity in each region, C_i^*, divided by the plasma activity is plotted on the ordinate.

Comparison between direct tissue counting and activity obtained from [^{18}F]FDG/PET

	Body weight (g)	Injected Dose MBq (mCi)	Recovery Rate*				
			Whole Brain	Cortex	Thalamus	Cerebellum	Brain Stem
Mean ± SD	241 ± 18	49.9 ± 10.5 (1.35 ± 0.28)	1.01 ± 0.17	0.90 ± 0.19	0.99 ± 0.04	0.84 ± 0.05	1.01 ± 0.24
Correlation coefficient between direct tissue counting and a ctivity obtained from PET images			0.71	0.73	0.99	0.96	0.43

Recovery rate was determined as (activity in MBq/cc in PET image acquired 55-60 min after injection of [^{18}F]FDG) / (activity in dissected tissue in MBq/cc). Nine studies were compared.

Conclusions Using [^{18}F]FDG/PET and ATLAS Small Animal Imaging

- **ATLAS is a high resolution & high sensitivity scanner.**
- **The plasma input function can be obtained because only FDG is present in blood. Now we use intraperitoneal injection with normalization (SUV).**
- **We do have the sensitivity for time activity curve time points, e.g., Patlak plots.**
- **The glucose metabolism rate agrees with previous autoradiographic studies.**

Benefits --- and --- Challenges

- Decreased use of animals
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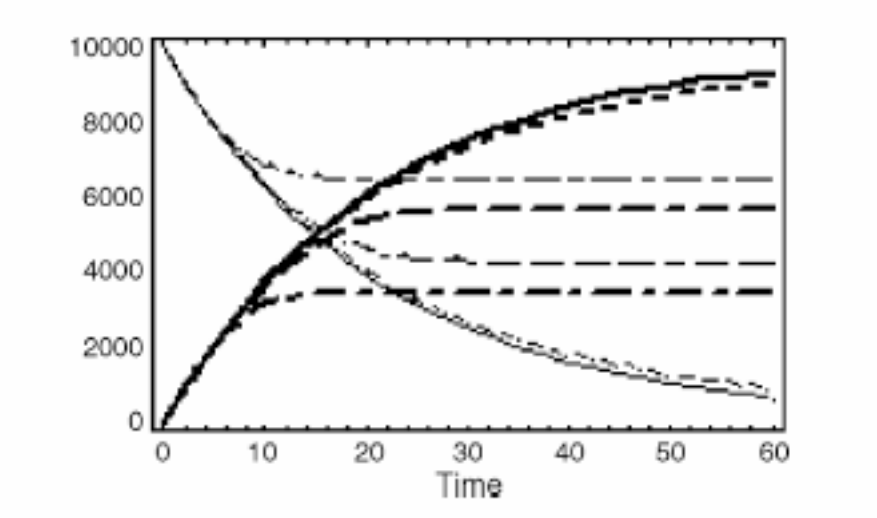
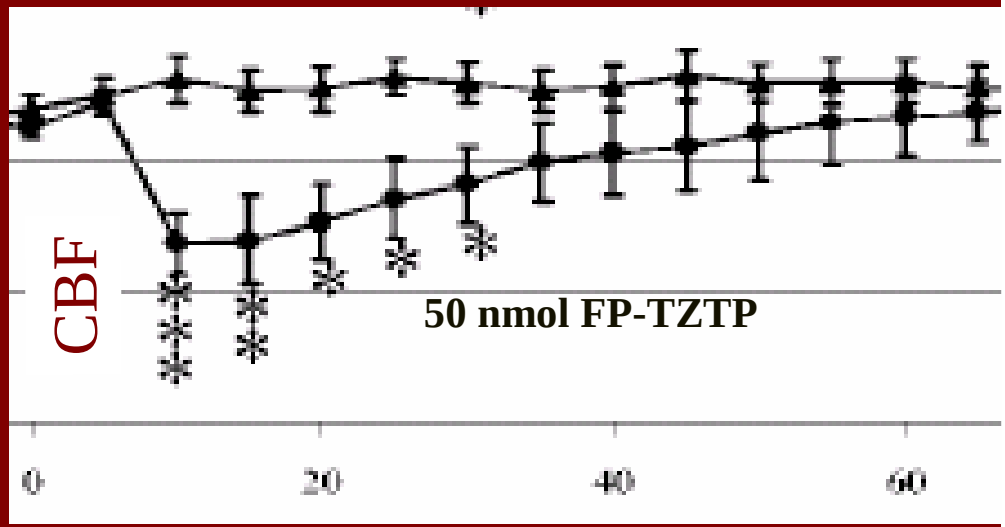
Mass and Dose (A) Requirements

- Rodents are 1/280 in size (0.250/70). < activity
- Radioactivity in human voxel (5 mm^3) must equal rodent voxel (1 mm^3) = x 625
- Assuming that scanner sensitivity and radioligand specific activity are the same and attenuation is 0.3 in humans then:
  - [Rat] = 280 x 0.3 [human] = 84 x more drug for equal dosing & resolution
- $A_{rat} = 0.3\ A_{human}$ not $0.04\ A_{human}$

Nuclear Medicine and Biology 31 (2004) 771–779

What is the problem with more mass per body weight for low density sites?





- More mass will affect kinetic order, but unless the saturation of the binding site is greater than 100%, the image will be comparable & today's computers can analyze the second order equation.
- More mass could affect physiology

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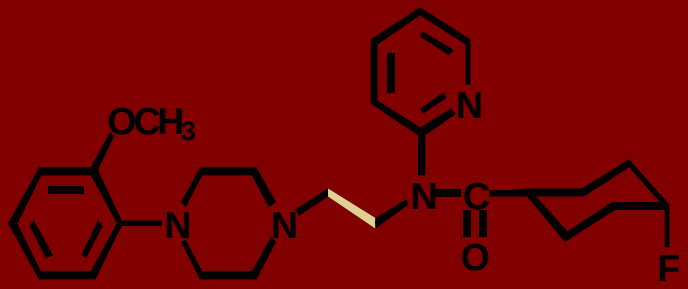
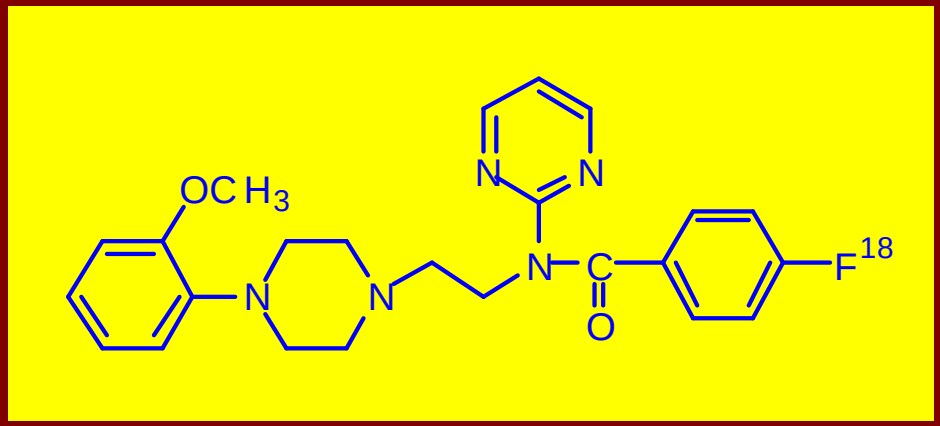
5-HT_{1A} Affinity for WAY100635 analogues

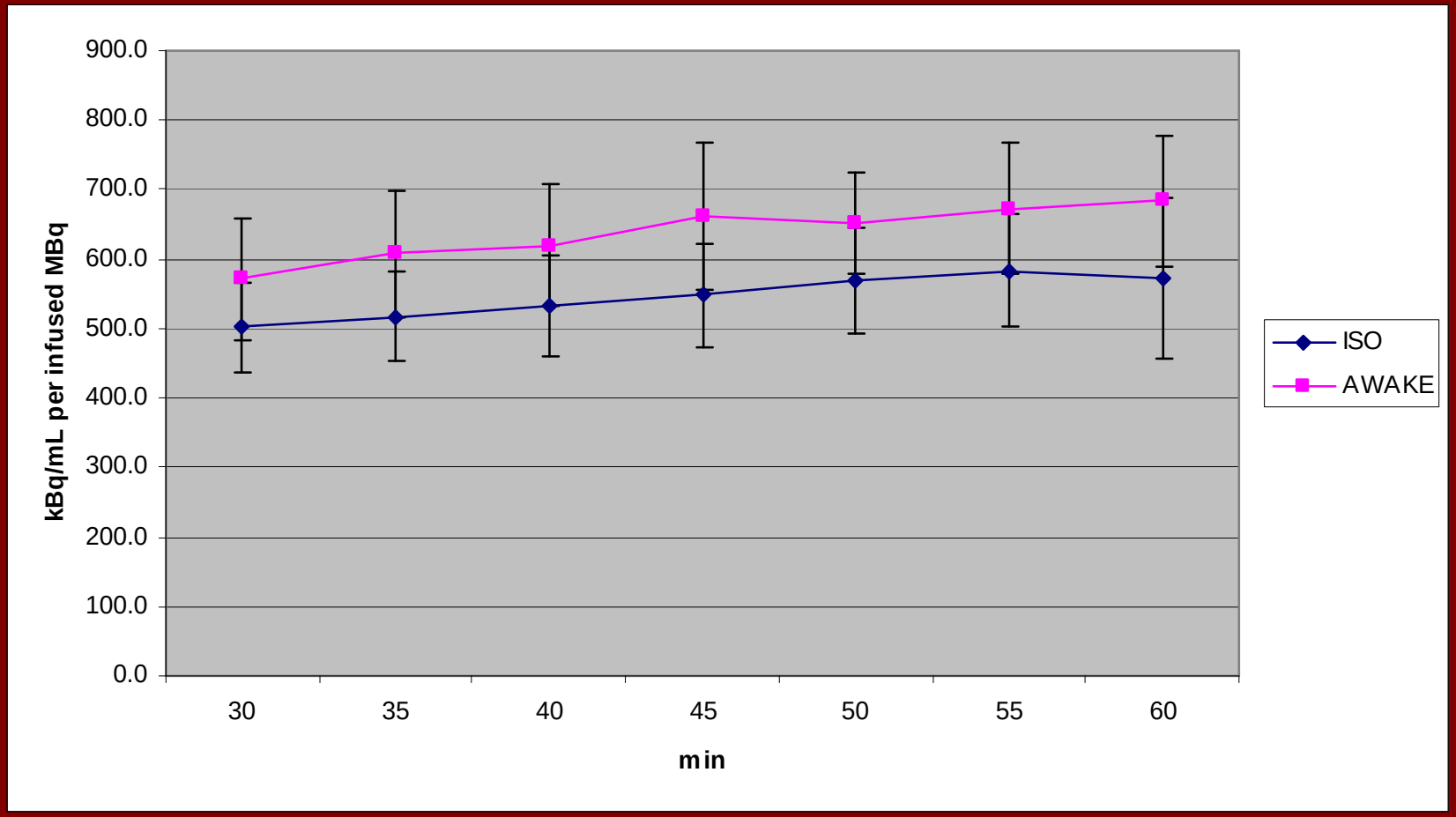
FC-WAY



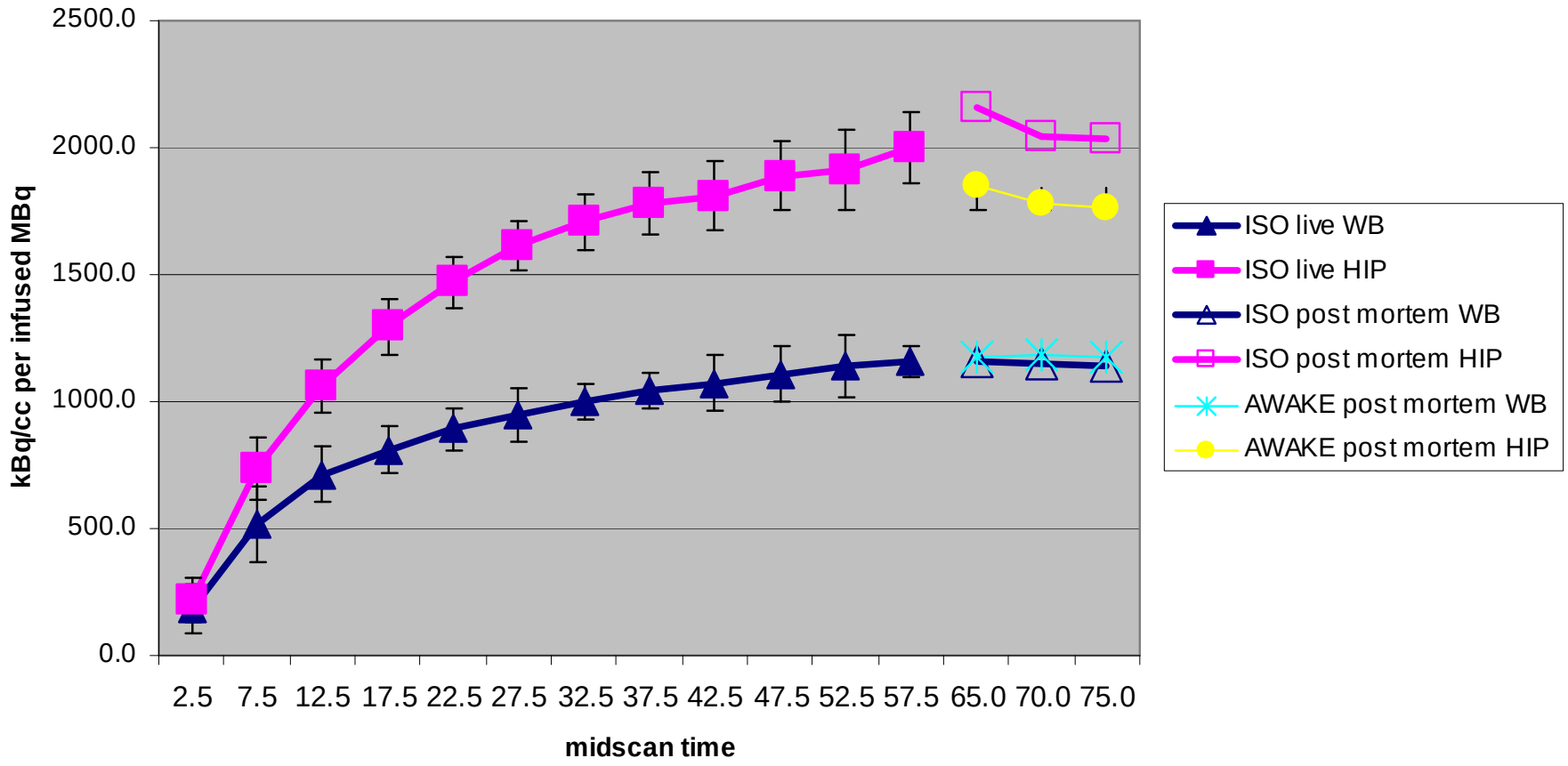
FP-WAY



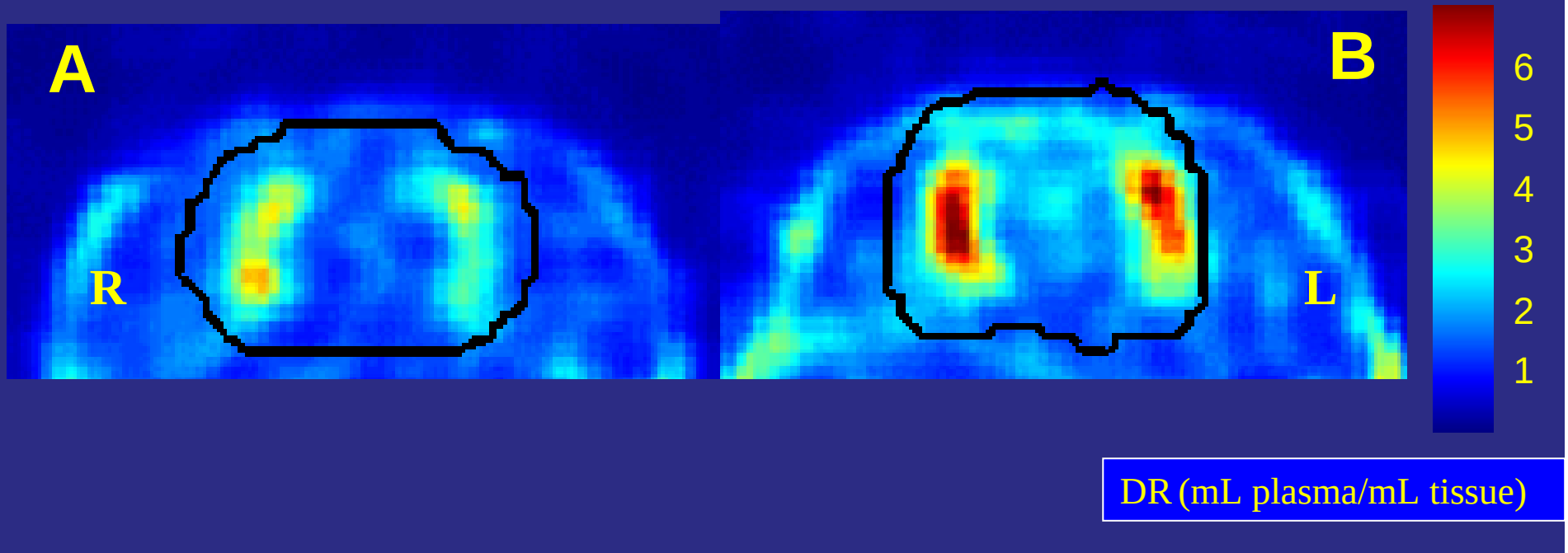
Arterial Plasma Time Courses Of [^{18}F]FPWAY Concentration Normalized To Activity Administered



Time courses of tissue radioactivity measured by live and postmortem PET scanning.

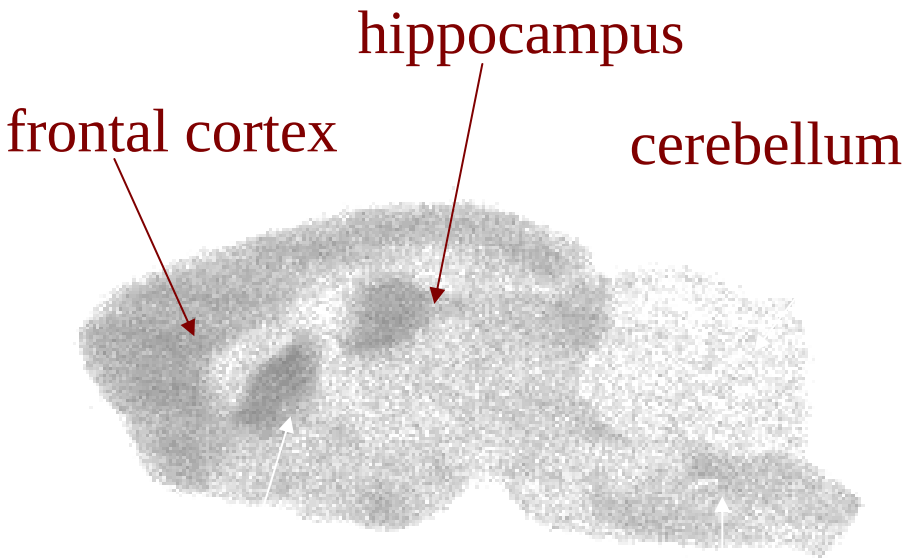


Brain:plasma distribution ratio determined from postmortem PET images of an awake (A) or isoflurane-anesthetized (B) rat

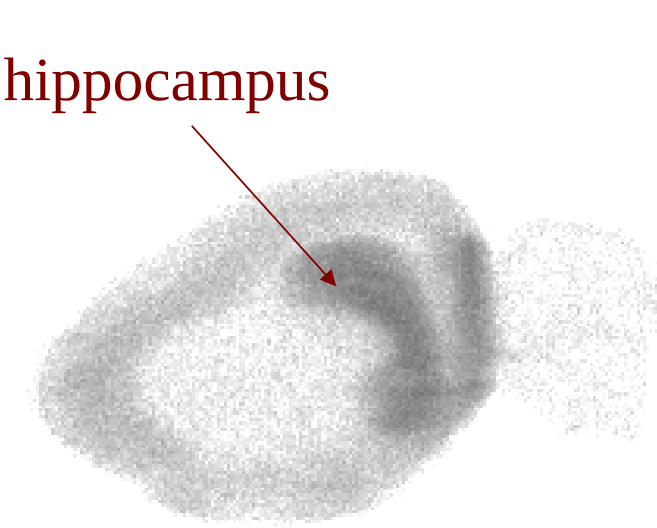


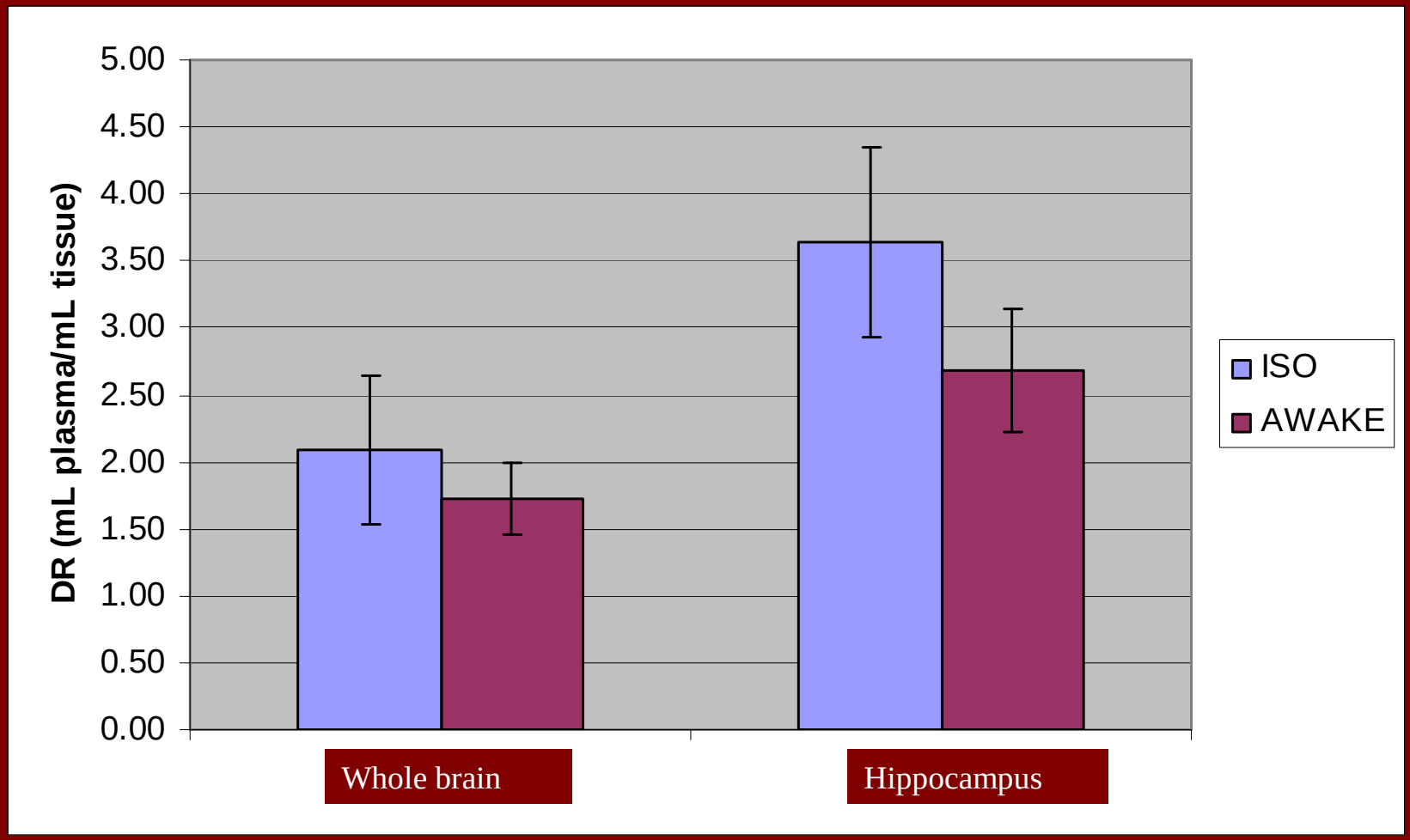
^{18}F autoradiographic images of sagittal sections from one rat. Sections are (A) medial, ~1 mm from the midline and (B) lateral, ~4 mm from the midline.

(A)

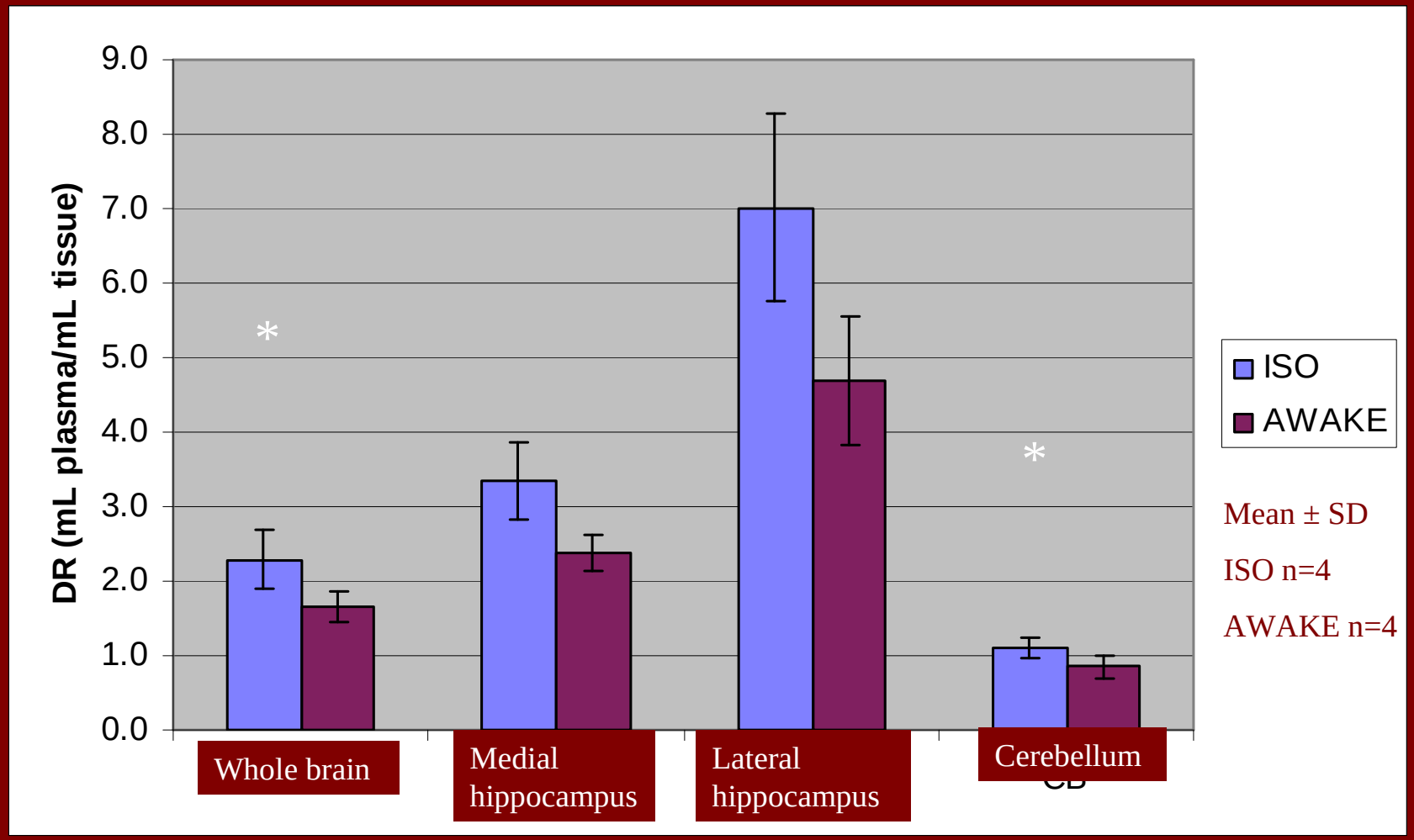


(B)




5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
DR (mL plasma/mL tissue)
ISO
AWAKE
Whole brain
Hippocampus

Confirmed by Autoradiography



Results

- DRs in the anesthetized animals were constant between 30 and 60 min, indicating near equilibrium between brain and plasma had been achieved by ~30 min.
- DRs determined from postmortem PET data were higher in the isoflurane-anesthetized rats by 24% (not significant) and 33% ($p=0.065$) in whole brain and hippocampus, respectively.
- DRs determined from autoradiographic data were greater in isoflurane-anesthetized rats in medial hippocampus, lateral hippocampus, and cerebellum by 33% ($p=0.054$), 63% ($p<0.01$), and 32% ($p<0.05$), respectively.

Conclusion

- [^{18}F]FPWAY could be an appropriate ligand for monitoring changes in receptor availability in the serotonergic system using a bolus/infusion paradigm. One possibility for higher DRs in anesthetized rats may be a reduction of endogenous 5-HT secretion under isoflurane anaesthesia.

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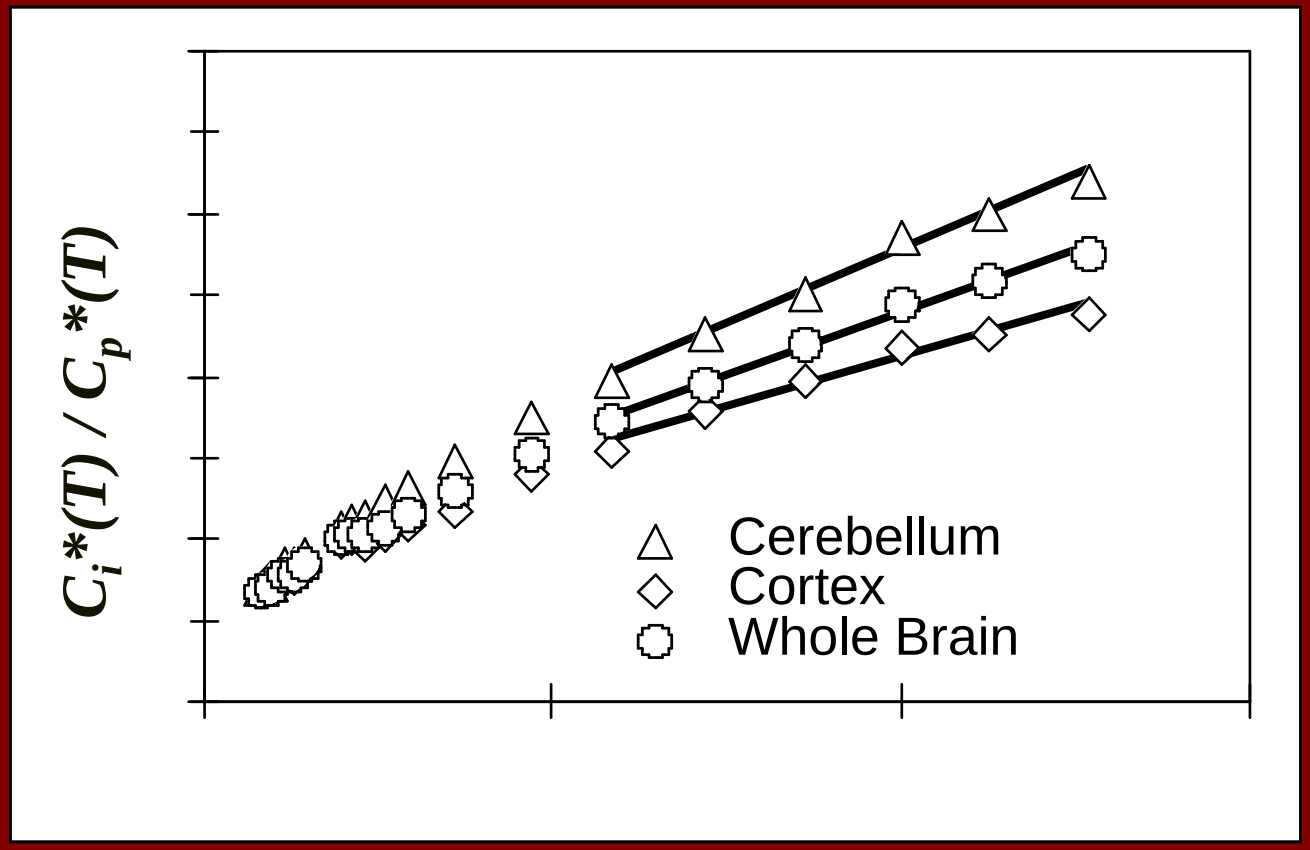
[^{18}F]FDG uptake in an anesthesized rat over 60 min.



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Conclusions for Small Animal Imaging

- For those radiotracers, e.g. FDG, for which the parent is the only species in blood, the procedure can be carried out as it is in clinical PET.
- For those radiotracers that clear the blood rapidly and both parent and metabolites are in blood, e.g. [^{18}F]FPWAY, the limited rodent blood volume makes bolus studies more difficult than infusion studies.
- Infusion studies are the paradigm of choice in small animal imaging for radiotracers that reach equilibrium rapidly.
- At present, no instant pretty pictures, but reliable biochemical parameters. WHAT IS IMPORTANT?

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