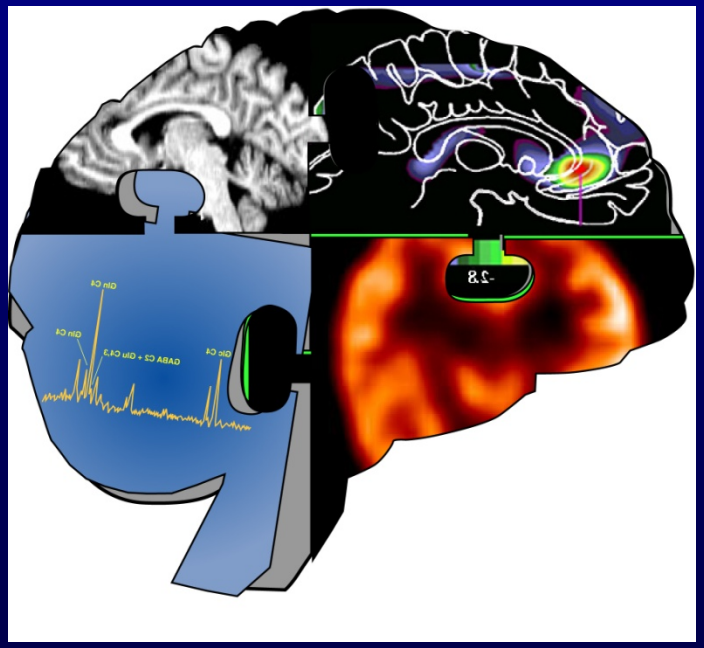
Positron Emission Tomographic (PET) Imaging of Efflux Transporters



Robert B. Innis, MD, PhD
Molecular Imaging Branch
NIMH

Outline of Talk

1. PET: high sensitivity and specificity
2. Many PET ligands already exist to measure density of transporters – e.g., dopamine transporter in Parkinson disease
3. P-gp: efflux transporter “protects” organs like brain and testis from some toxins and drugs
4. [^{11}C]loperamide: avid P-gp substrate but has radiometabolite; measures function
5. [^{11}C]desmethyl-loperamide (dLop): metabolite is better than parent
6. After P-gp blockade, [^{11}C]dLop has high brain uptake that is dependent on flow
7. [^{11}C]dLop in humans: no brain uptake at baseline and slightly increased by P-blockade

Imaging of neuroreceptors by PET

Isotope production
[^{11}C ^{18}F ^{13}N ^{15}O]

Cyclotron

$^{11}CO_2$

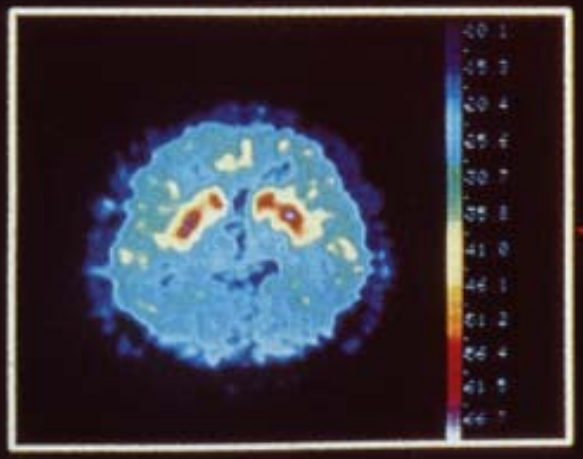
Radio chemistry

Precursor

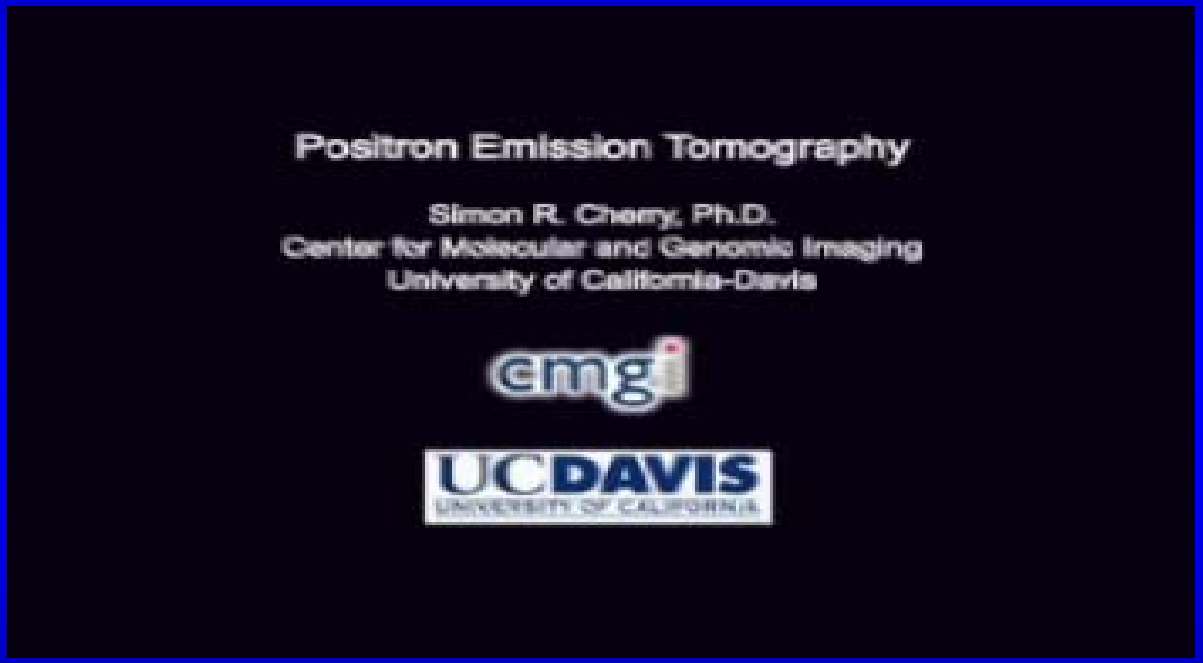
^{11}C-ligand

Positron camera

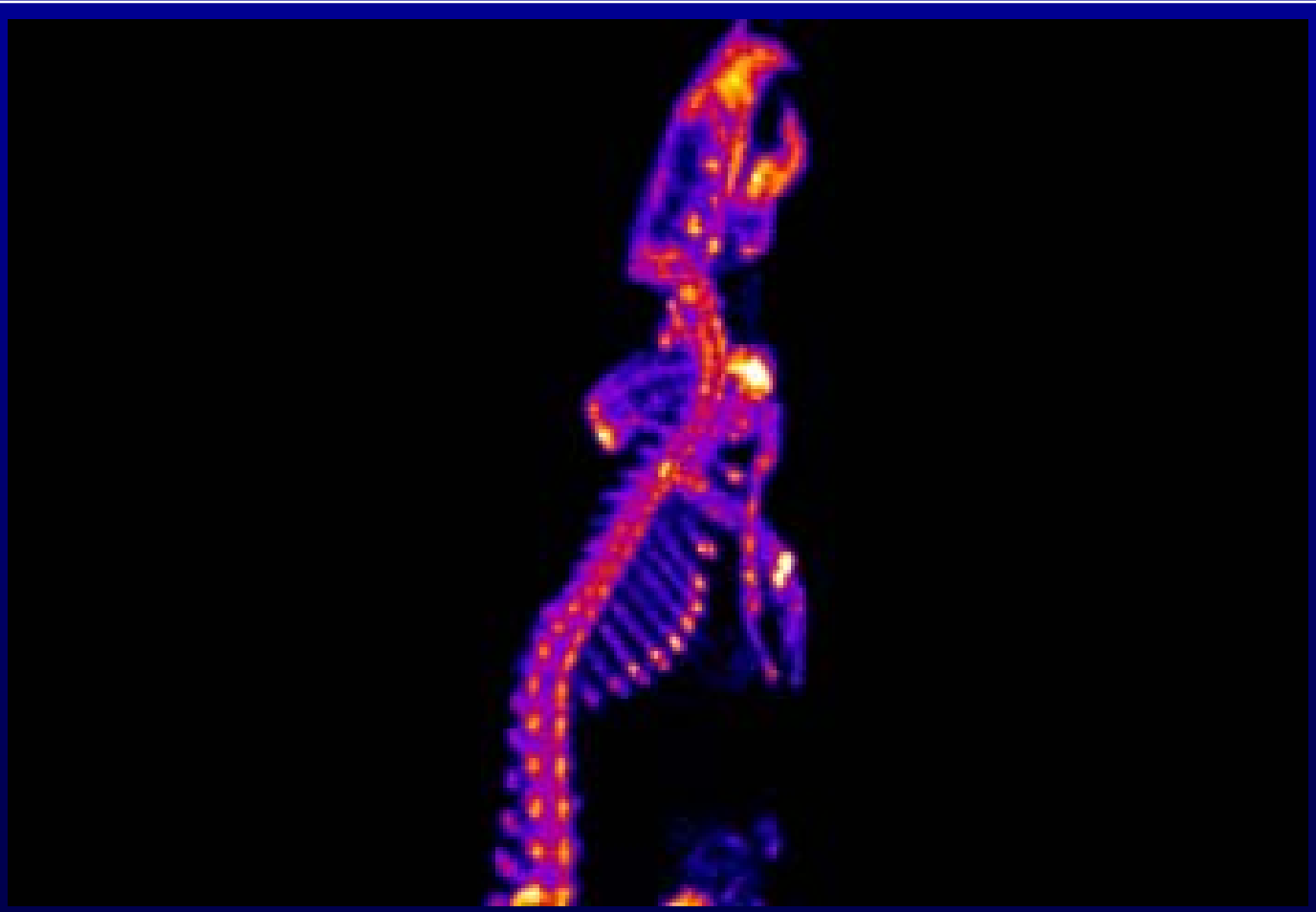
Image of
ligand distribution
in brain



Positron Emission Tomography



NIH Rodent PET Camera
^{18}F bone uptake rat



Developed By: Mike Green & Jurgen Seidel

PET vs. MRI

	PET	MRI
Spatial Resolution	2 – 6 mm	<< 1 mm
Sensitivity	10^{-12} M	10^{-4} M
Temporal Resolution	minutes	<1 sec

Radionuclide (^{11}C): high sensitivity

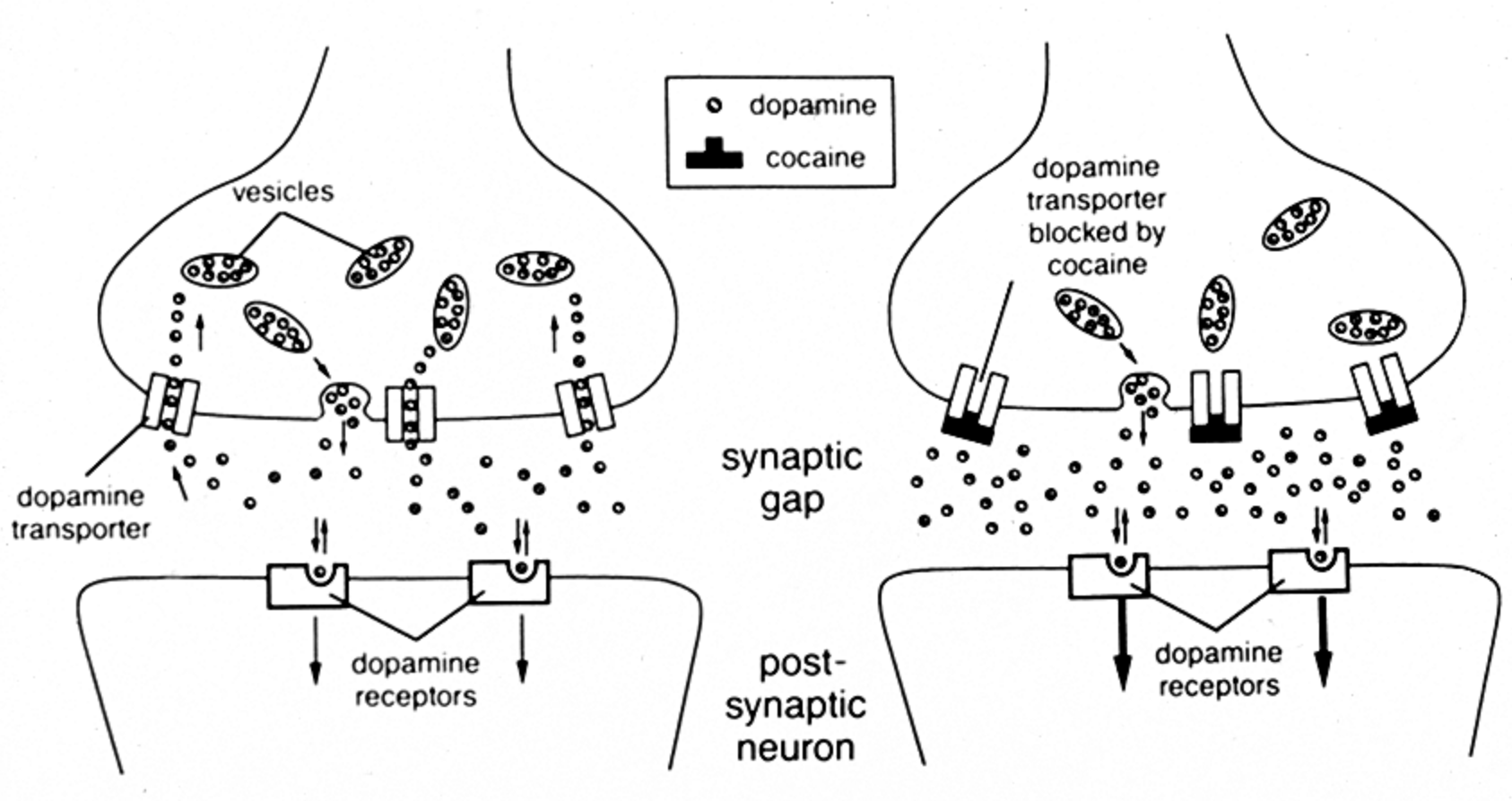
Ligand (raclopride): high selectivity

Radioligand [^{11}C]raclopride: high sensitivity & selectivity

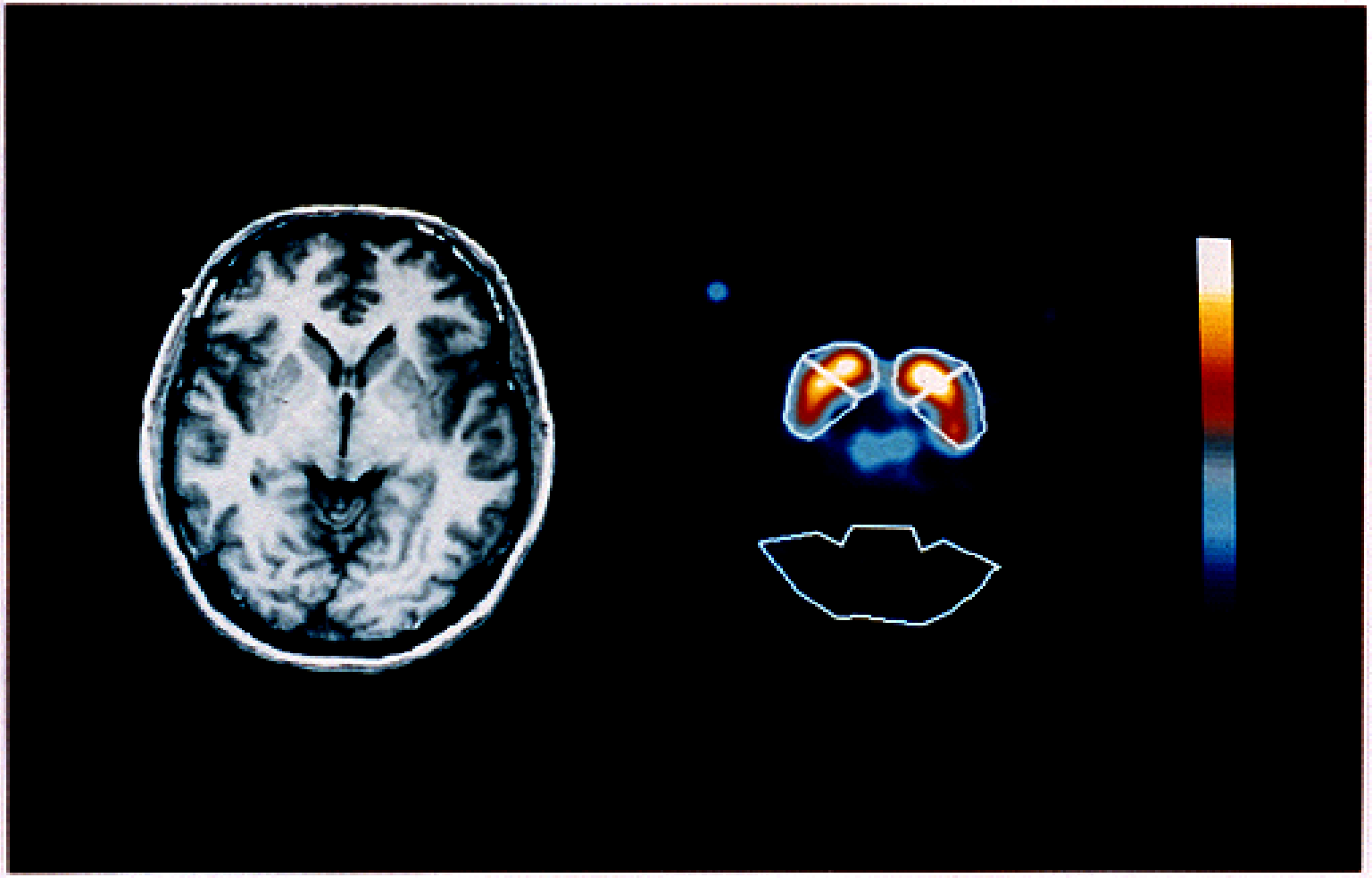
Radioligand = Drug + Radioactivity

1. **Drug administered at tracer doses**
   a) No pharm effects
   b) Labels <1% receptors
   c) Labeled subset reflects entire population
2. **Radioligand disposed like all drugs**
   a) Metabolism & distribution
3. **Radiation exposure**

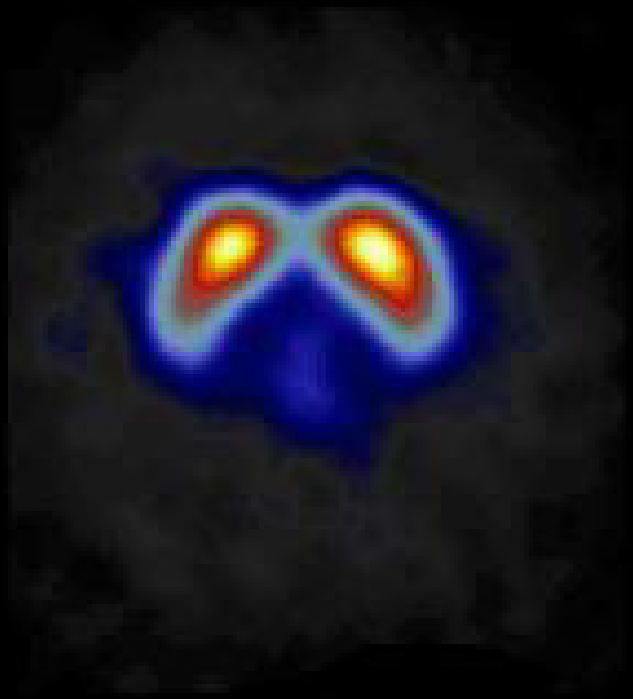
Dopamine Transporter: Located on DA Terminals Removes DA from Synapse



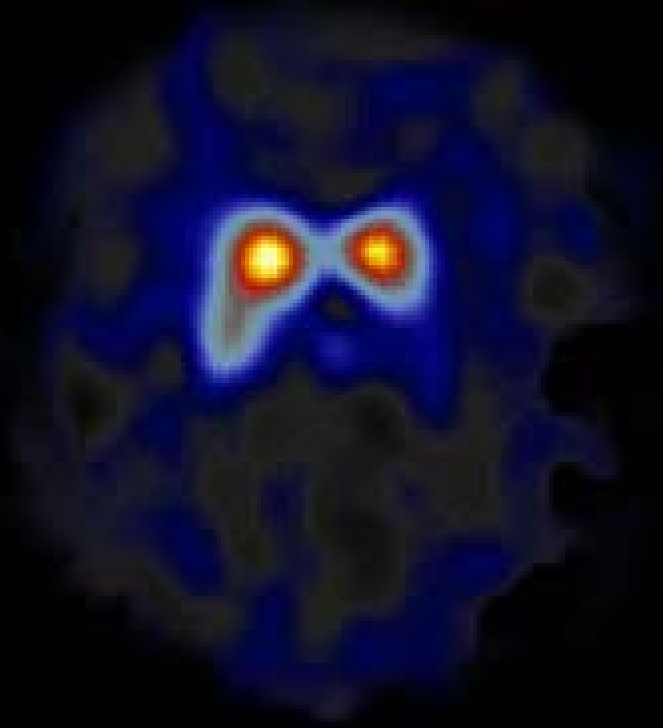
SPECT Imaging of Dopamine Transporter in Caudate and Putamen of Human Brain



^{123}I-β-CIT Dopamine Transporter SPECT: Decreased in Parkinson's Disease

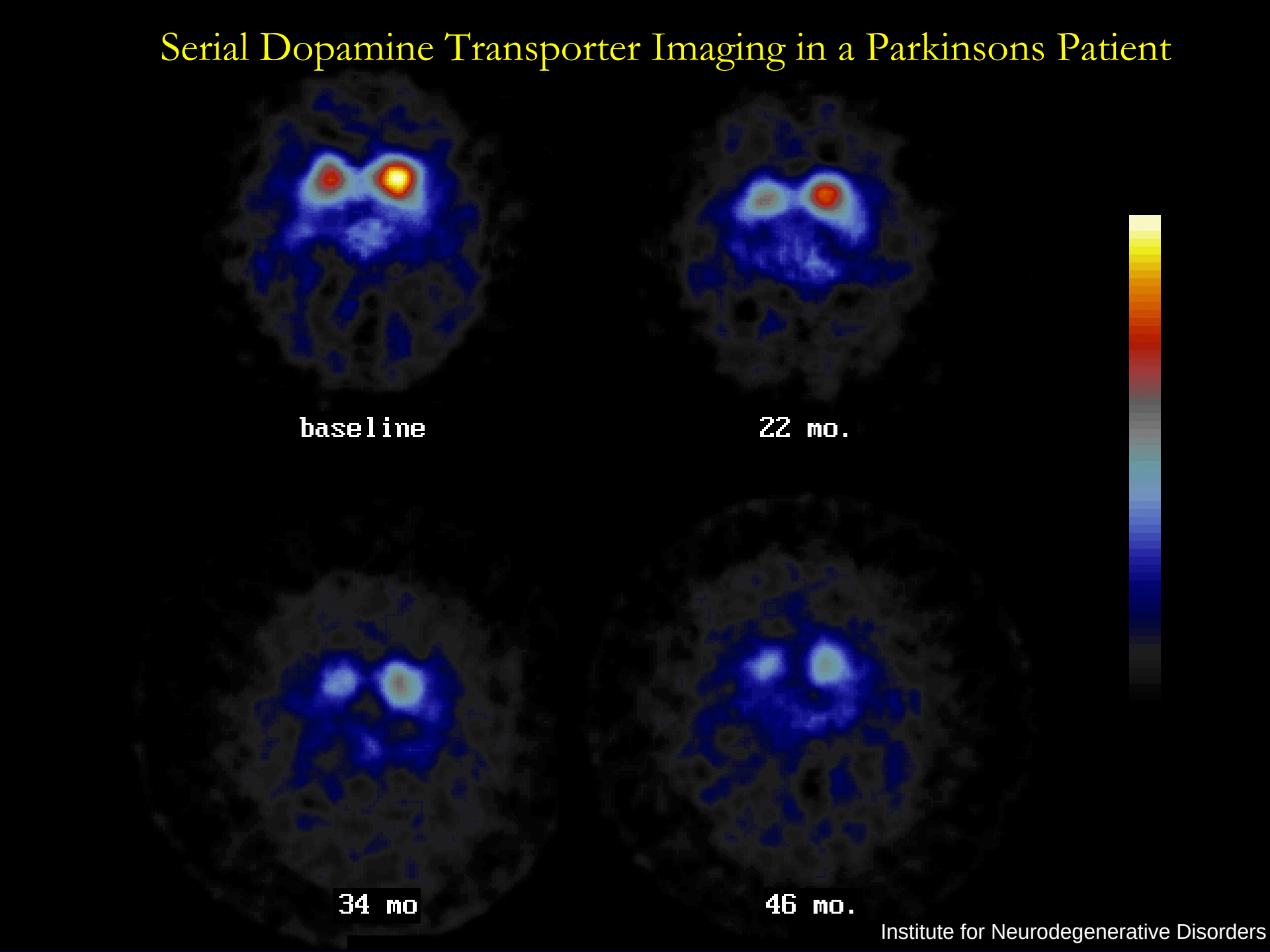


Healthy



Parkinson
Stage 1

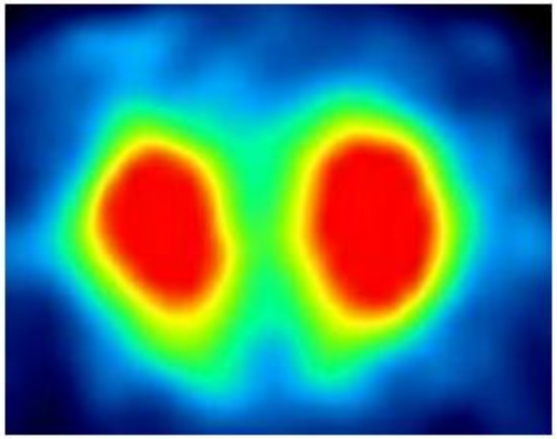
Serial Dopamine Transporter Imaging in a Parkinsons Patient



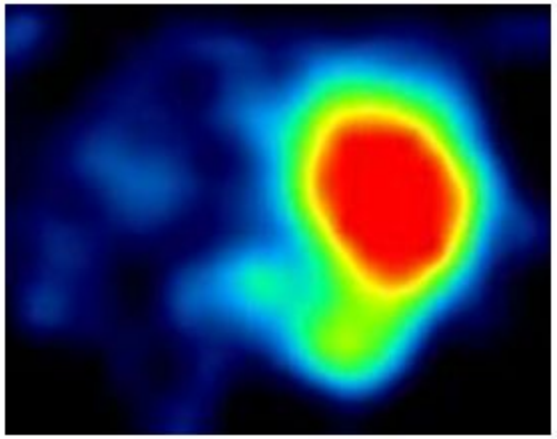
Institute for Neurodegenerative Disorders

PET Imaging to Monitor Embryonic Stem Cell Treatment of "Parkinson Disease" in Rats

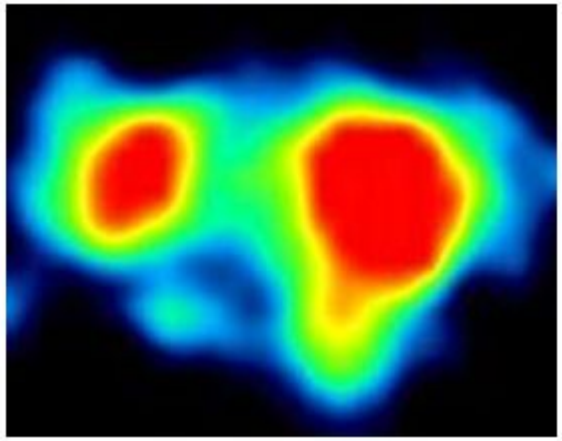
Normal



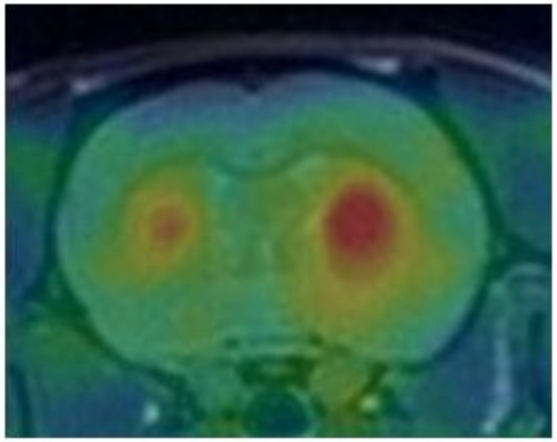
Unilateral Lesion



Embryonic Stem Cells



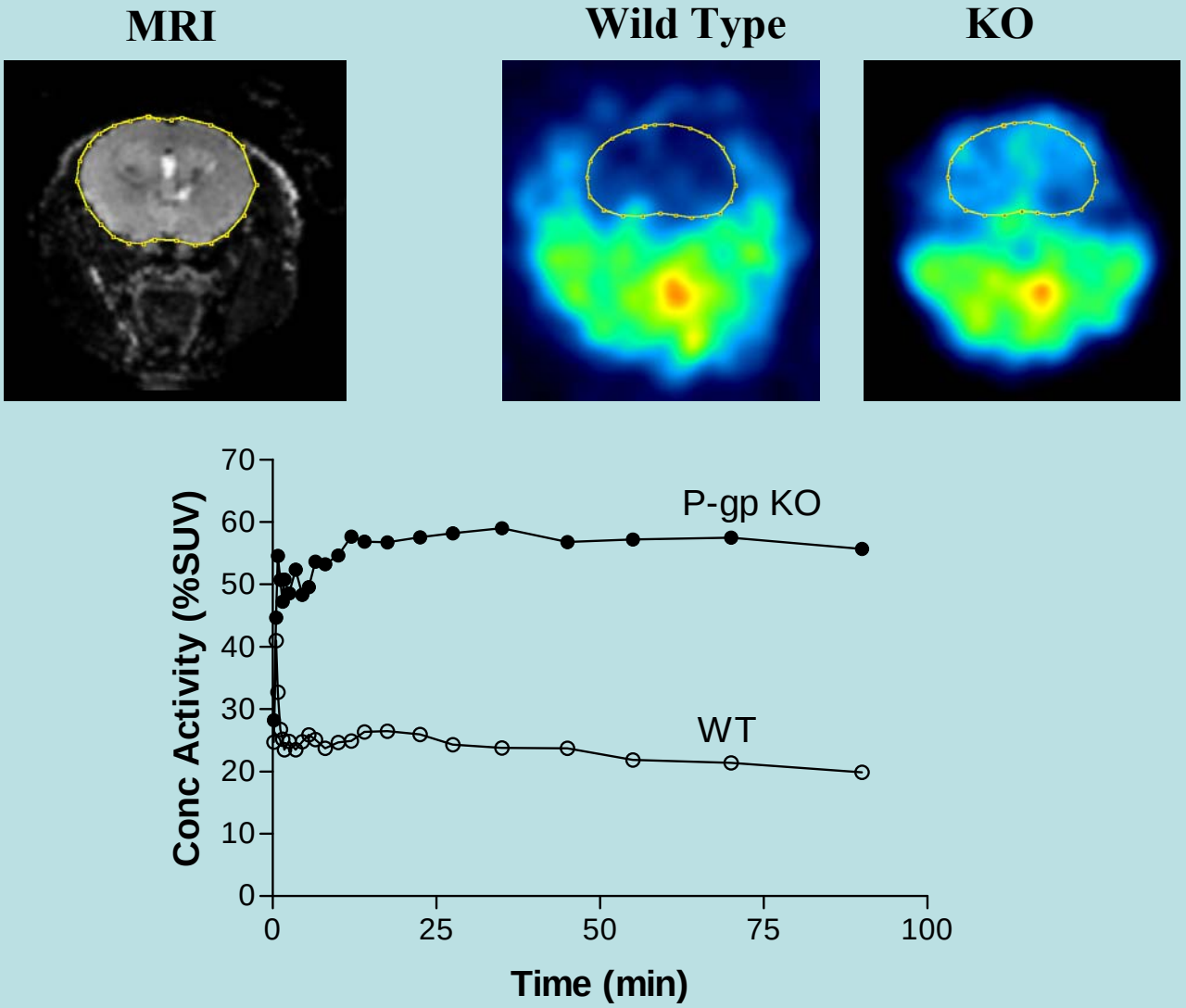
PET & MRI



P-glycoprotein (P-gp) Efflux Transporter

1. Transports drugs out of cells in many locations – e.g., brain and testes
2. Specific component of blood-brain barrier
3. Loperamide (Imodium®) is a potent opiate that acts on gut to slow motility – but no actions in brain.
4. Over expressed in 40% of tumors resistant to chemotherapy

[^{11}C]Loperamide: brain uptake much higher in P-gp KO than in wild type mice



Injection of [^{11}C]Loperamide in P-gp Knockout and Wild Type Mice

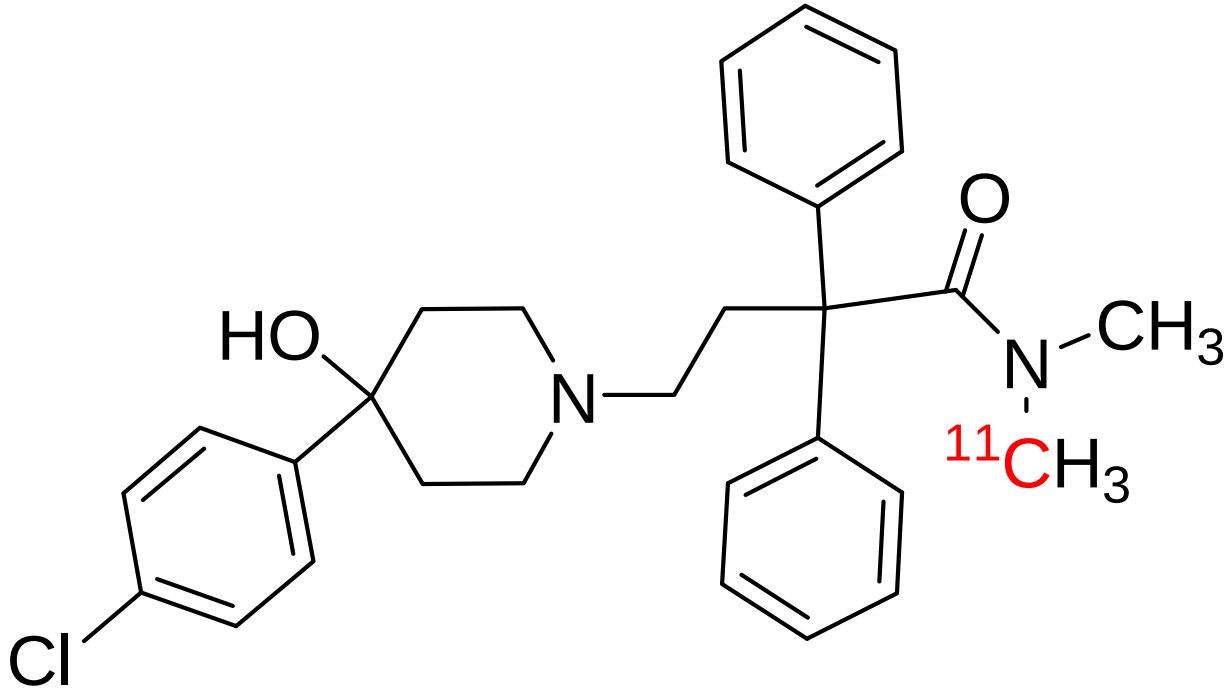
	Brain		
	Concentration (%SUV)		% Brain Activity
Radiochemical Species	KO	WT	KO
[^{11}C]Loperamide	25	2	**50%**
[^{11}C]dLop	12	1	24%
[^{11}C]Metabolites	14	11	26%
Total	51	14	100%

Five P-gp KO and five WT mice were killed 30 min after injection of [^{11}C]loperamide.

PROBLEM of [^{11}C]Loperamide

Radiometabolite (desmethyl) enters brain

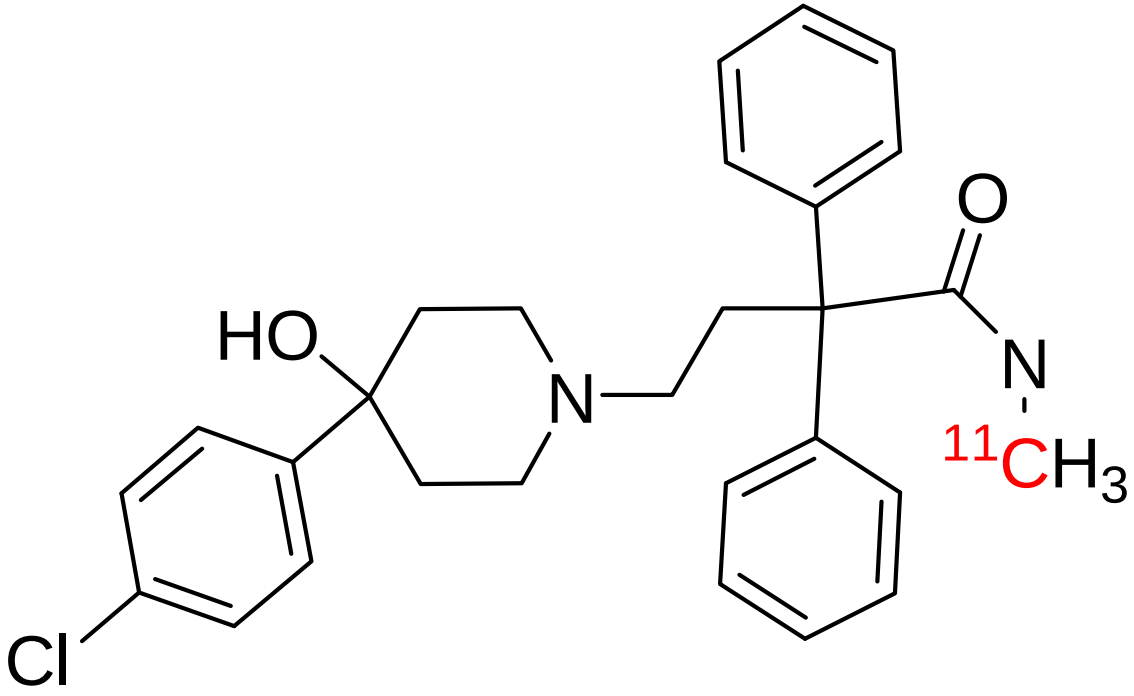
[^{11}C]Loperamide



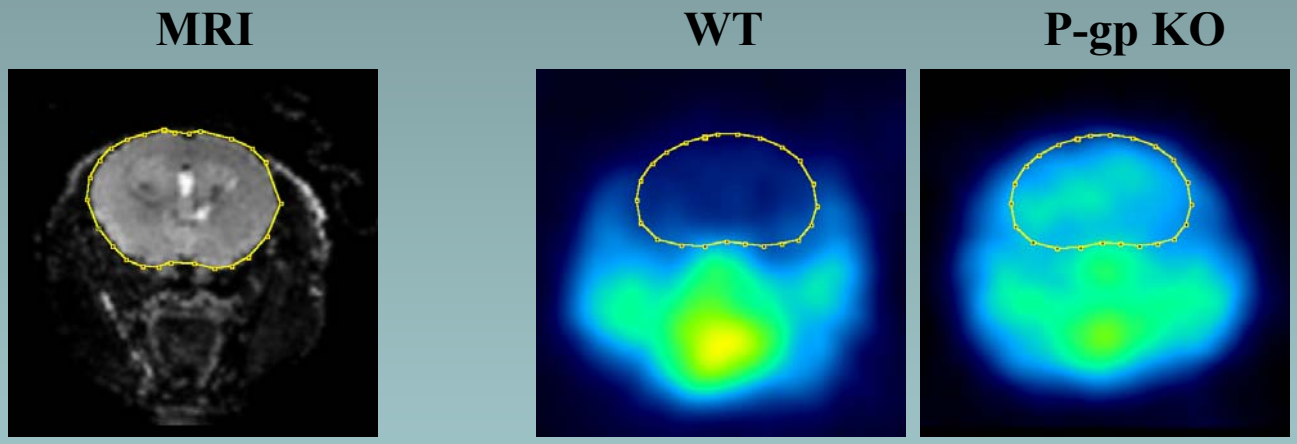
Solution: Remove the nonradioactive methyl group

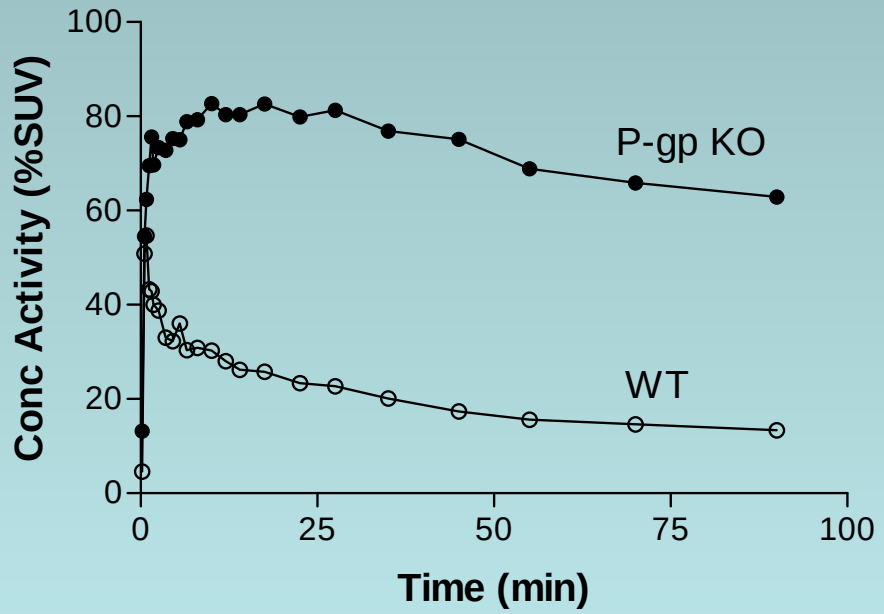
[^{11}C]Desmethyl-loperamide: Better radioligand?

Demethylation product does not enter brain



[^{11}C]dLop: brain uptake much higher in P-gp KO than in wild type mice



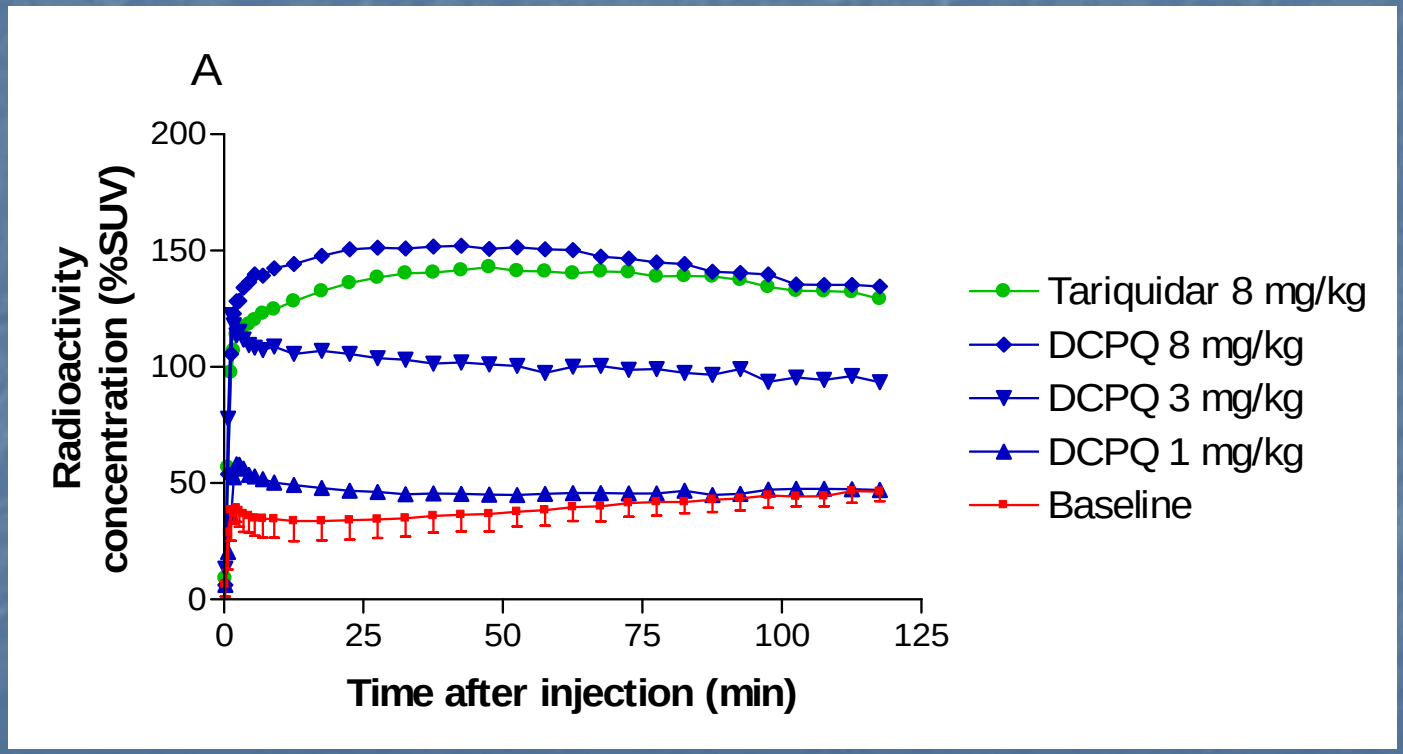


Injection of [^{11}C]*N*-desmethyl-Loperamide in P-gp Knockout and Wild Type Mice

	Brain		
	Concentration (%SUV)		% Brain Activity
Radiochemical Species	KO	WT	KO
[^{11}C]dLop	36	2	**92 %**
[^{11}C]Metabolites	3	3	8 %
Total	39	5	100 %

Three P-gp KO and three WT mice were killed 30 min after i.v. injection of [^{11}C]dLop.

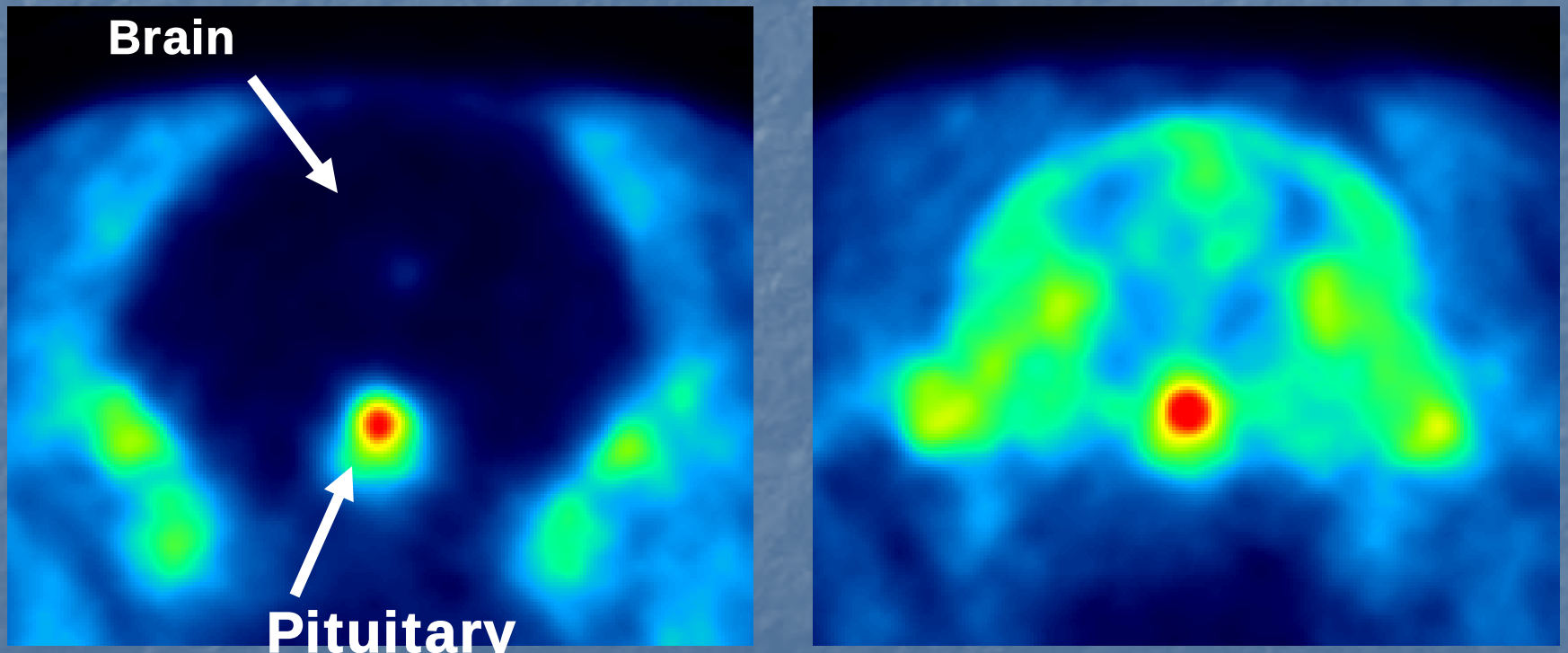
DCPQ or Tariquidar Increases Brain Uptake of Radioactivity in Monkey Given [^{11}C]Loperamide



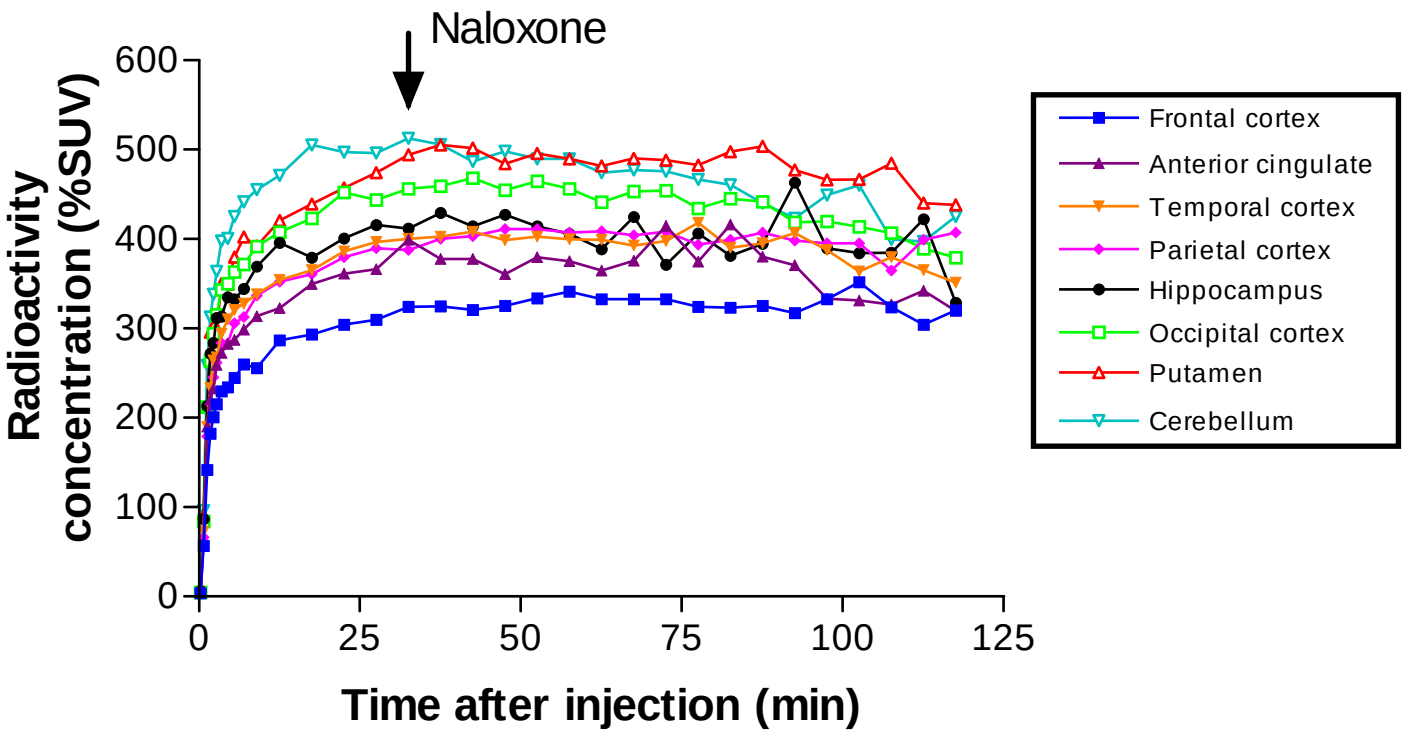
[^{11}C]dLop in Monkey Brain

Baseline

P-gp blocked

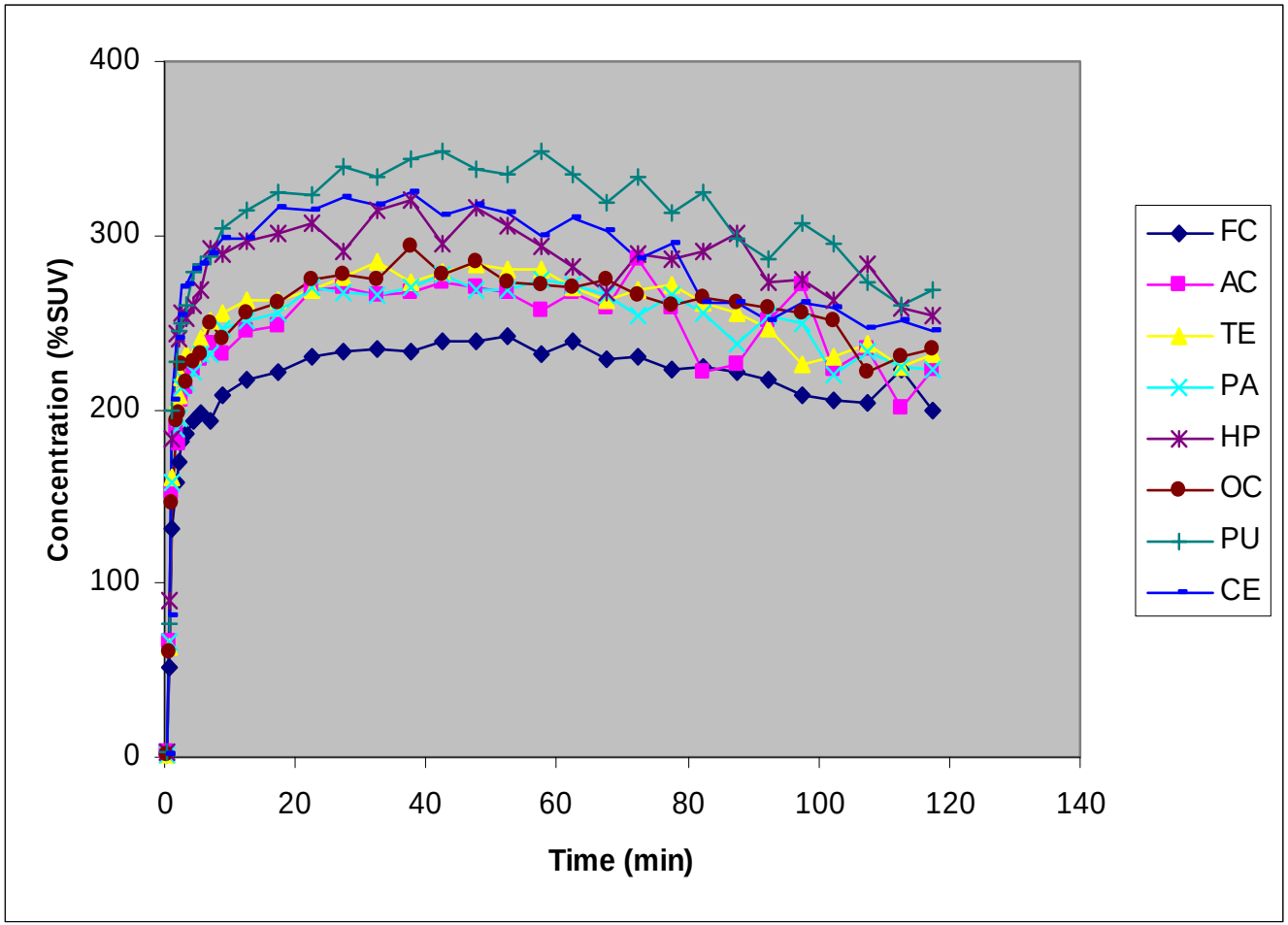


[^{11}C]dLop in Monkey Brain:
Radioligand does not bind to opiate receptors



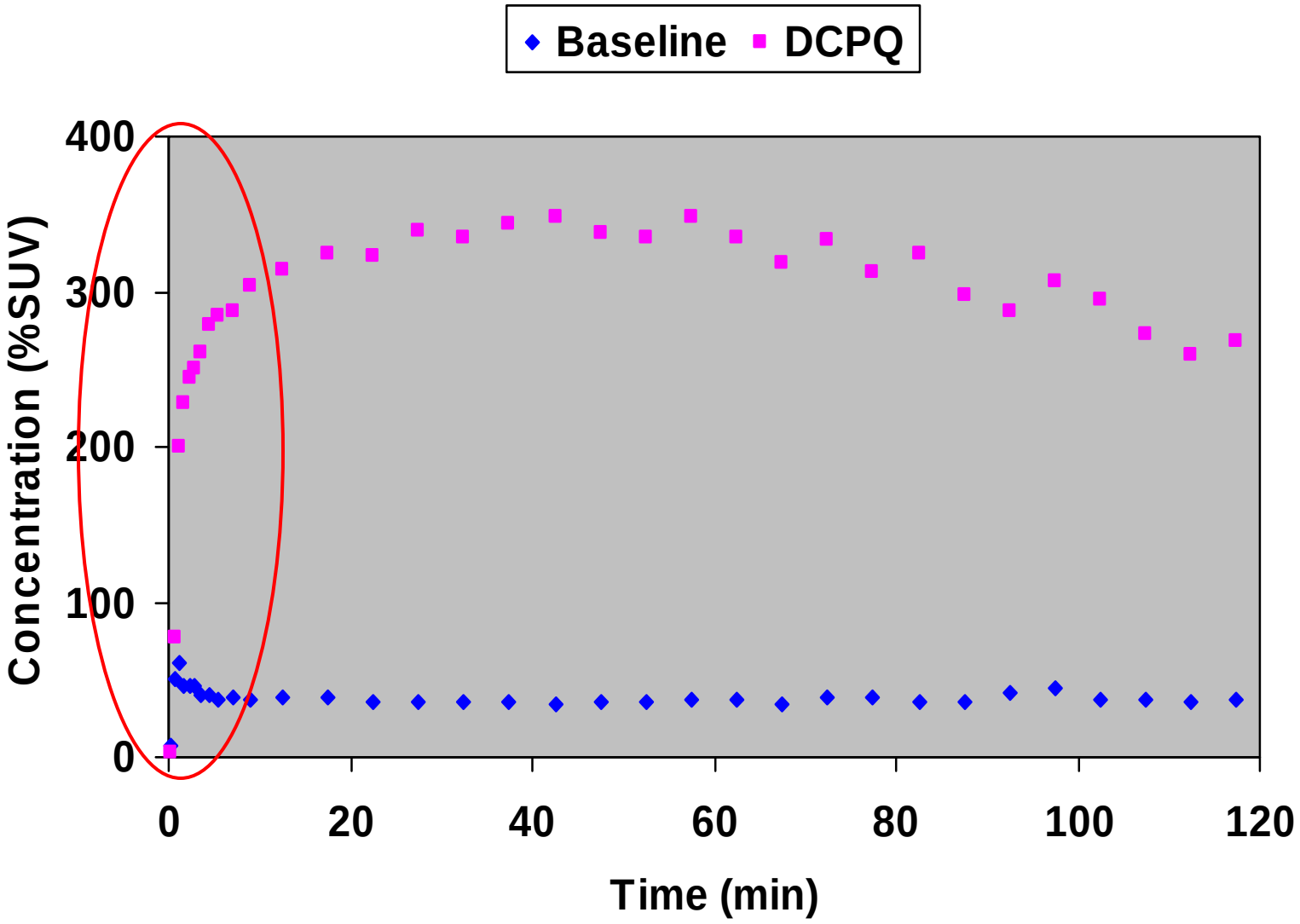
DCPQ 16 mg/kg, Naloxone 5 mg/kg (30 min after injection)

Is P-gp function uniformly distributed in brain?

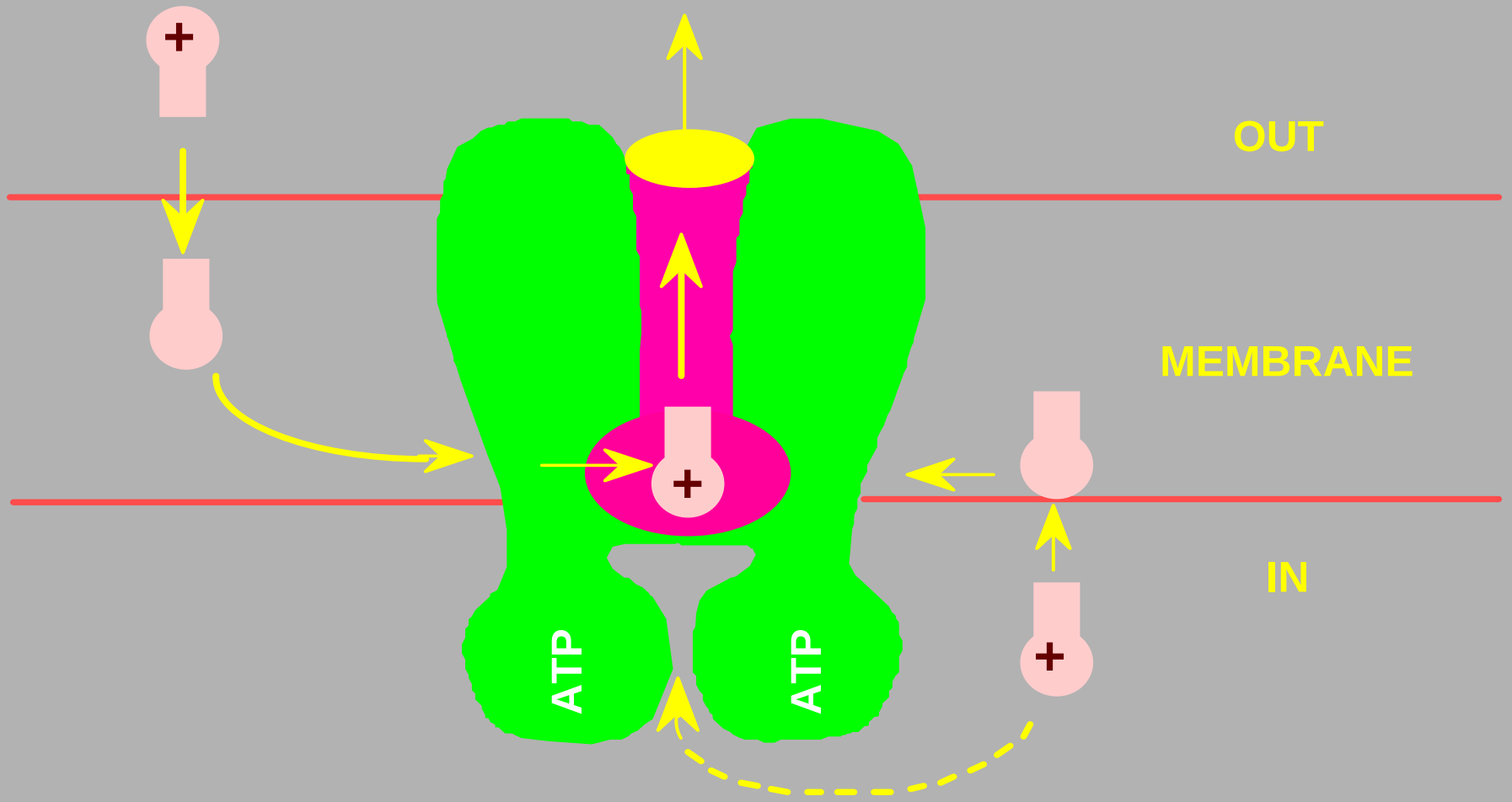


FC=Frontal Cortex, AC=Anterior Cingulate Gyrus, TE=Temporal Cortex , PA=Parietal Cortex, HP=Hippocampus, OC= Occipital Cortex, PU=Putamen, CE=Cerebellum

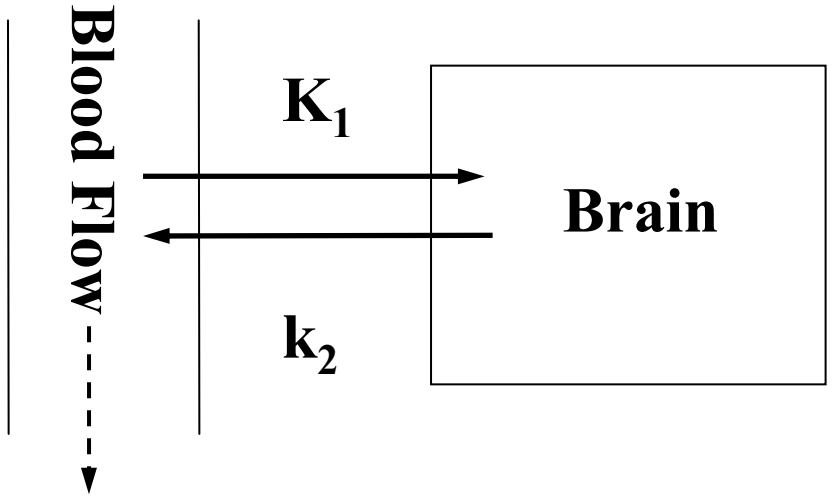
Brain uptake is rapid and probably dependent on blood flow.



P-glycoprotein removes lipophilic substrates directly from the plasma membrane



Brain uptake depends on blood flow and single pass extraction.



K_1 = rate brain entry

K_1 = flow · extraction

$K_1 = F \cdot E$

Example

Flow of drug 100 µg per min

Extraction is 2%

K_1 = 2 µg per min

Single Pass Extraction of [^{11}C]dLop >50%

1) Measure K_1 from brain and plasma data of [^{11}C]dLop
2) Measure blood flow with [^{15}O]H_2O
3) Calculate Extraction (E)

$$E = \frac{K_1}{F}$$

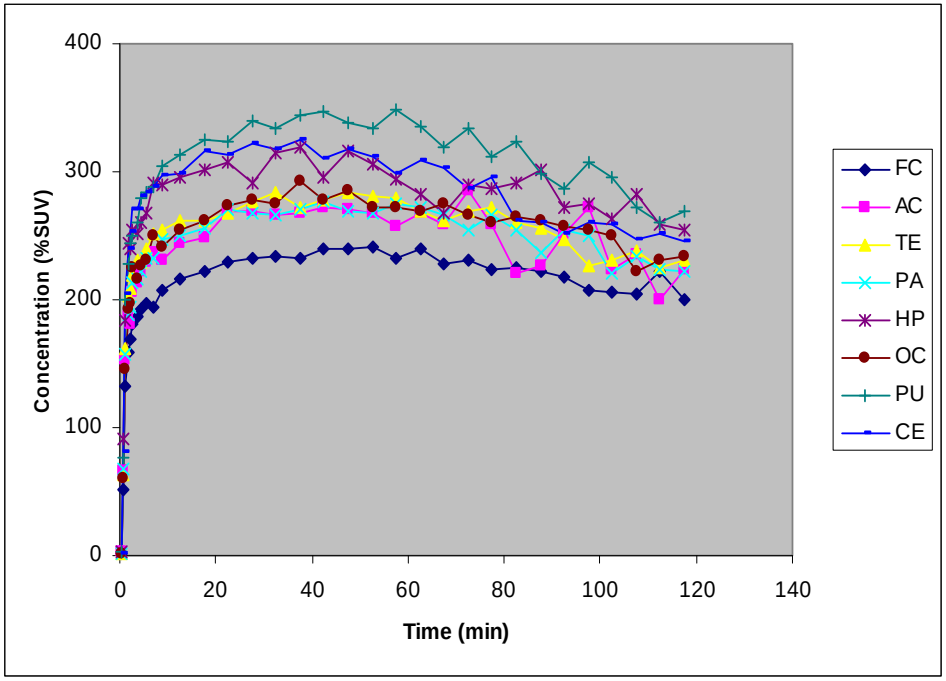
K_1 > 0.25 mL per cm^3 per min

F = 0.5 mL per cm^3 per min

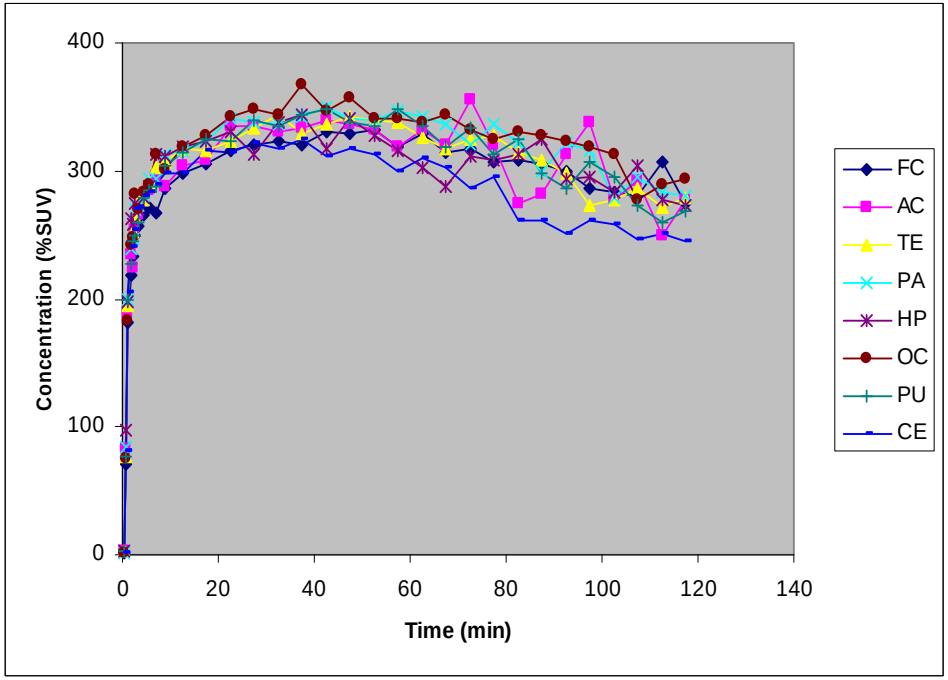
E > 0.5 = 50%

After correction for relative blood flow, [^{11}C]dLop uptake is uniform among brain regions

No Flow Correction



With Flow Correction



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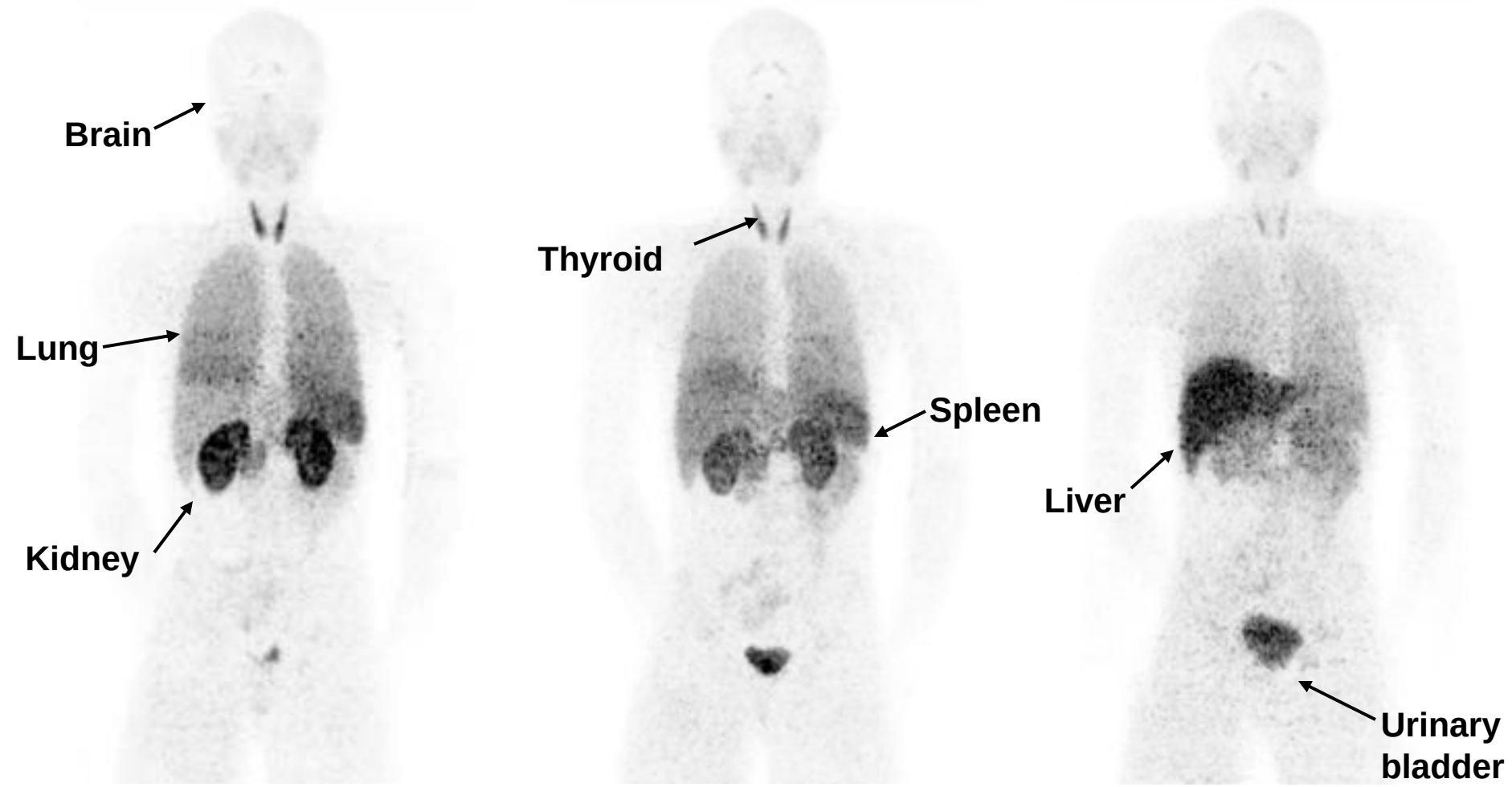
Conclusions

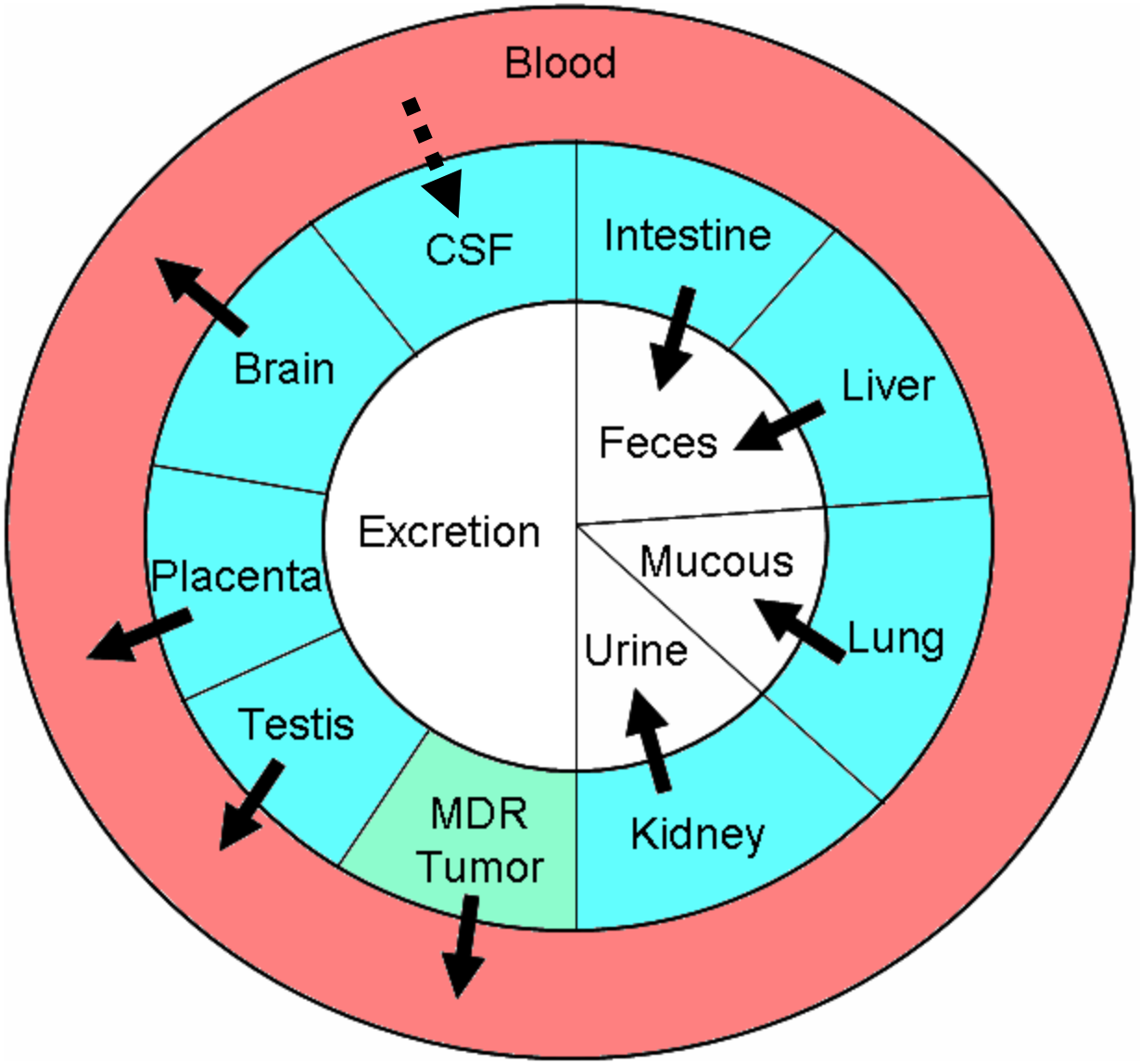
1. **[^{11}C]dLop: avoids metabolite problem of [^{11}C]loperamide**
2. **After P-gp blockade, single pass uptake of [^{11}C]dLop into brain is high and, therefore, shows dependence on blood flow**

Implies function of P-gp at baseline is rapid and has high capacity

[^{11}C]dLop: Distribution of radioactivity in healthy male

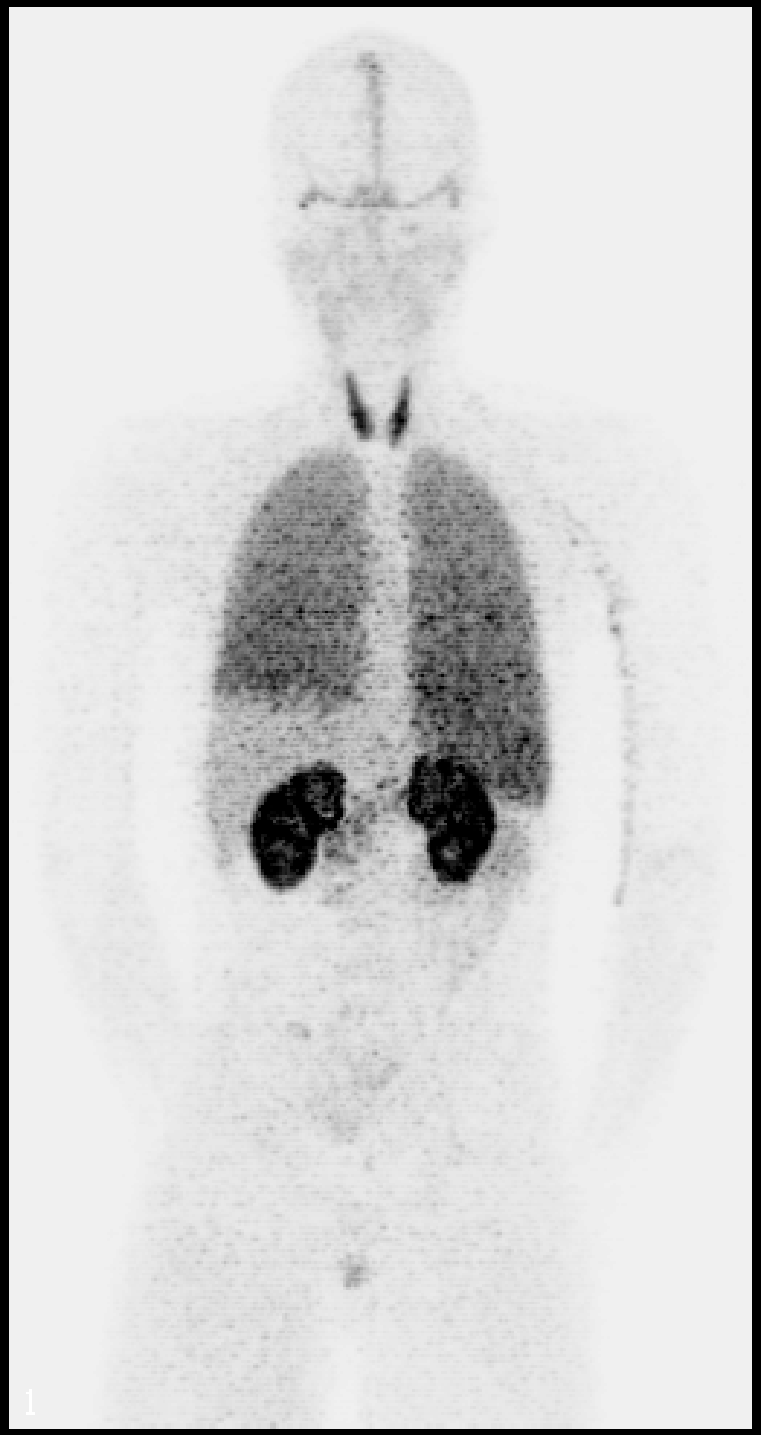


P-gp Transport in Human Body

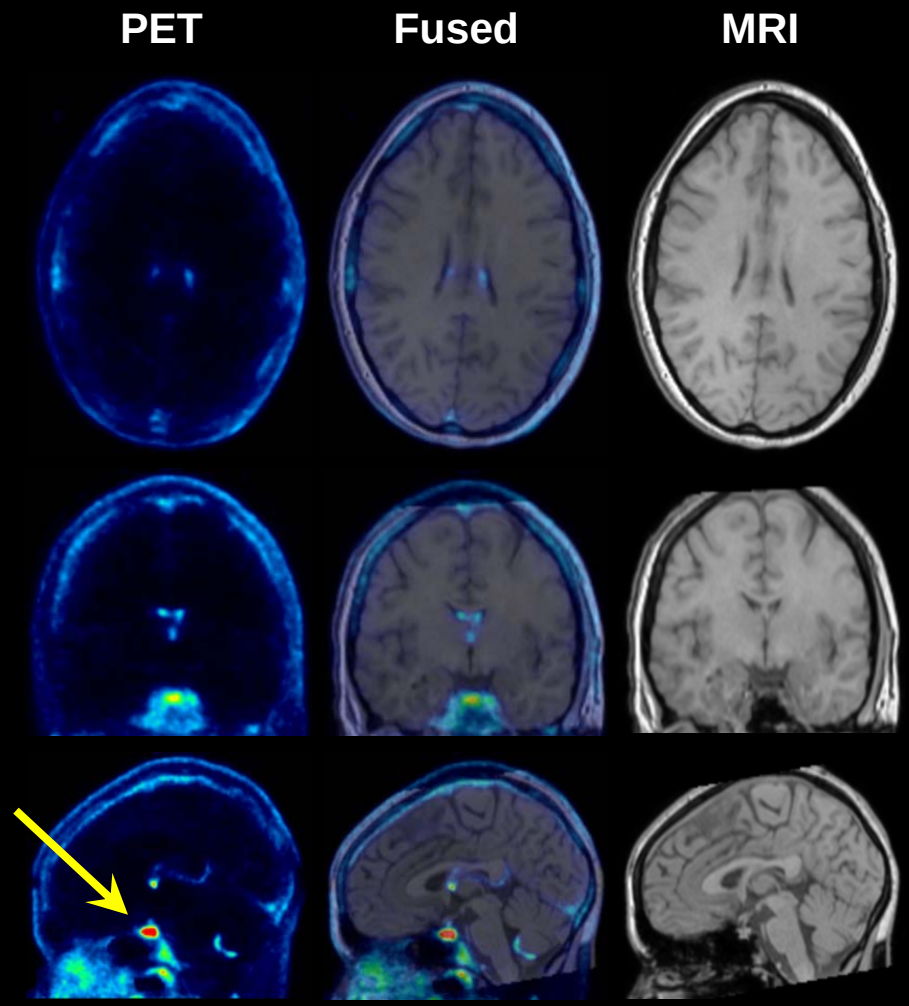


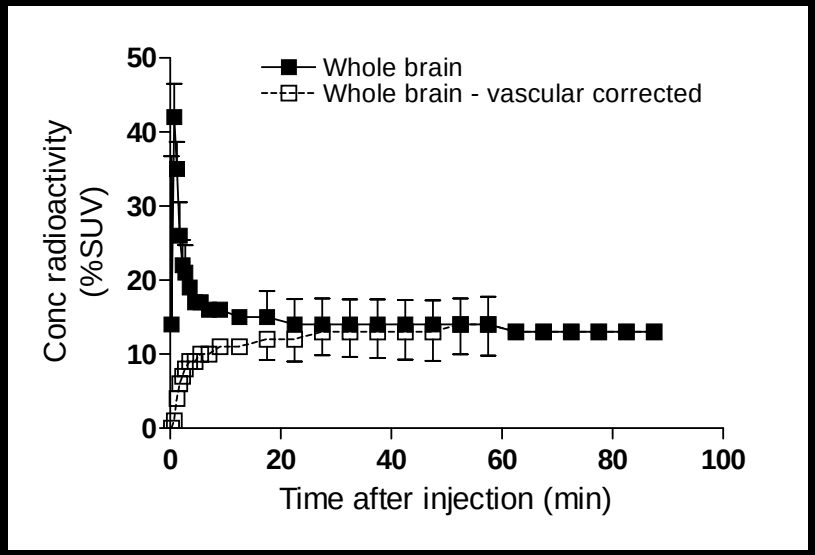
Direction of transport

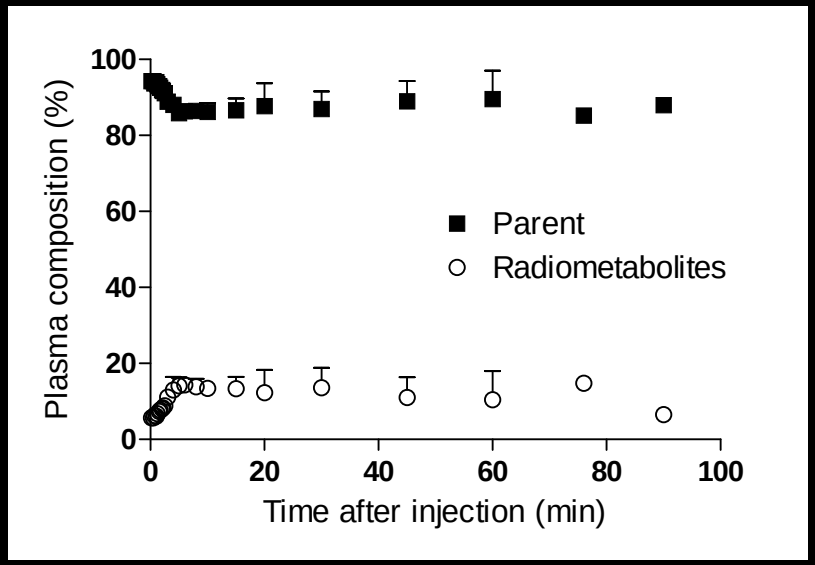
Summed early images (0 – 3 min) show blood pool.

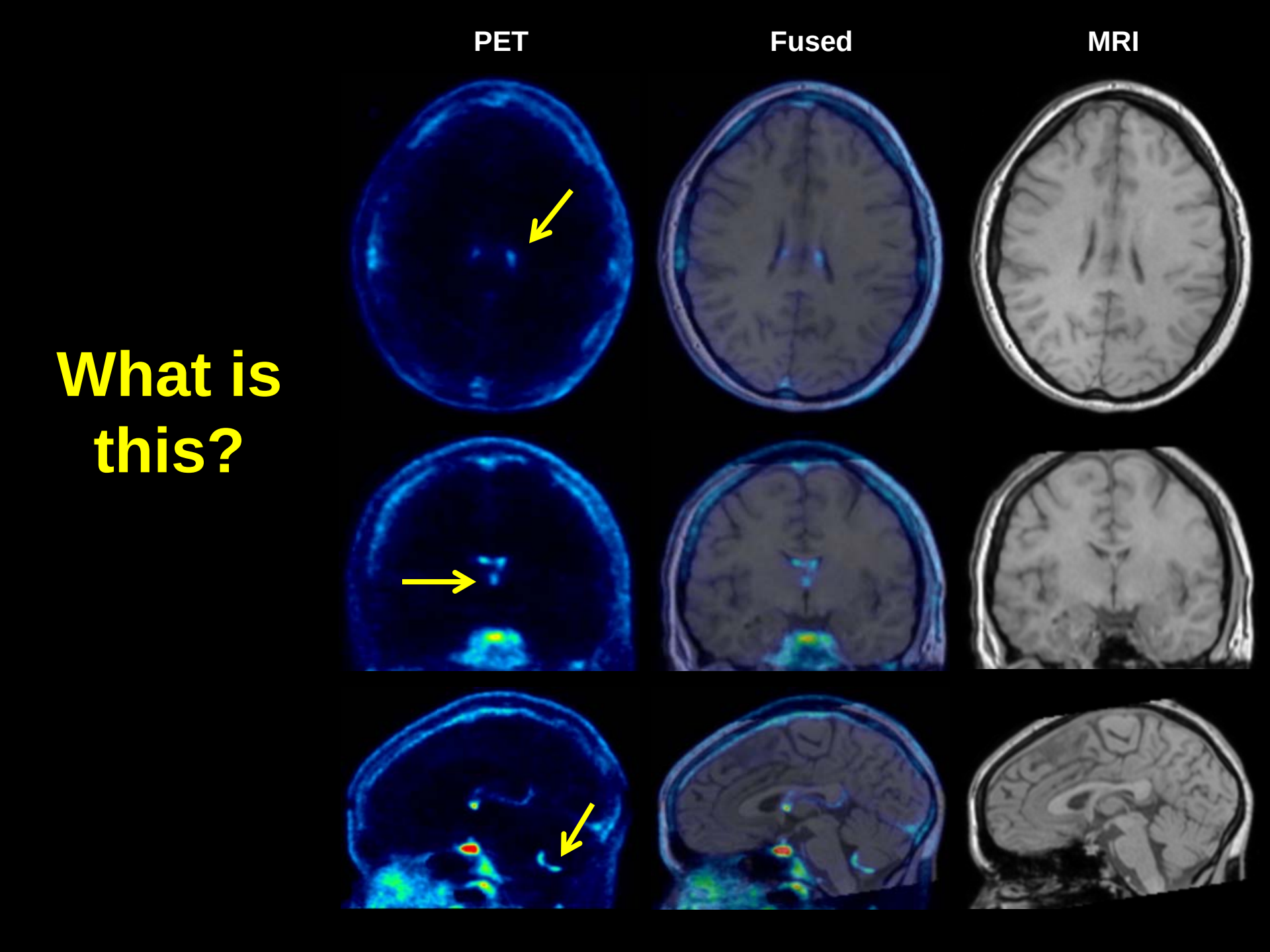


Minimal brain uptake of [^{11}C]dLop in healthy human brain

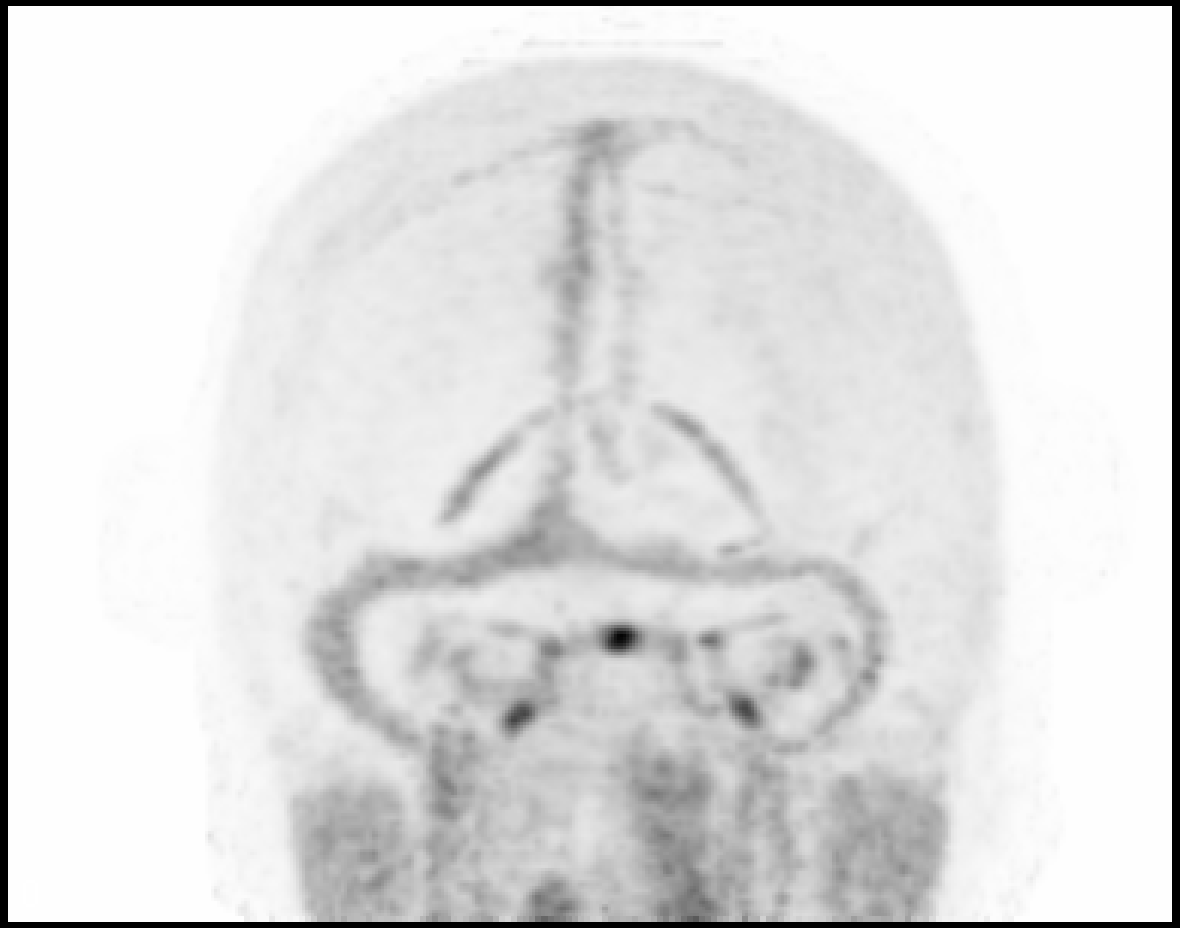







PET
Fused
MRI
What is this?

Extended summed images (0 – 10 min) show blood pool and tissue accumulation.



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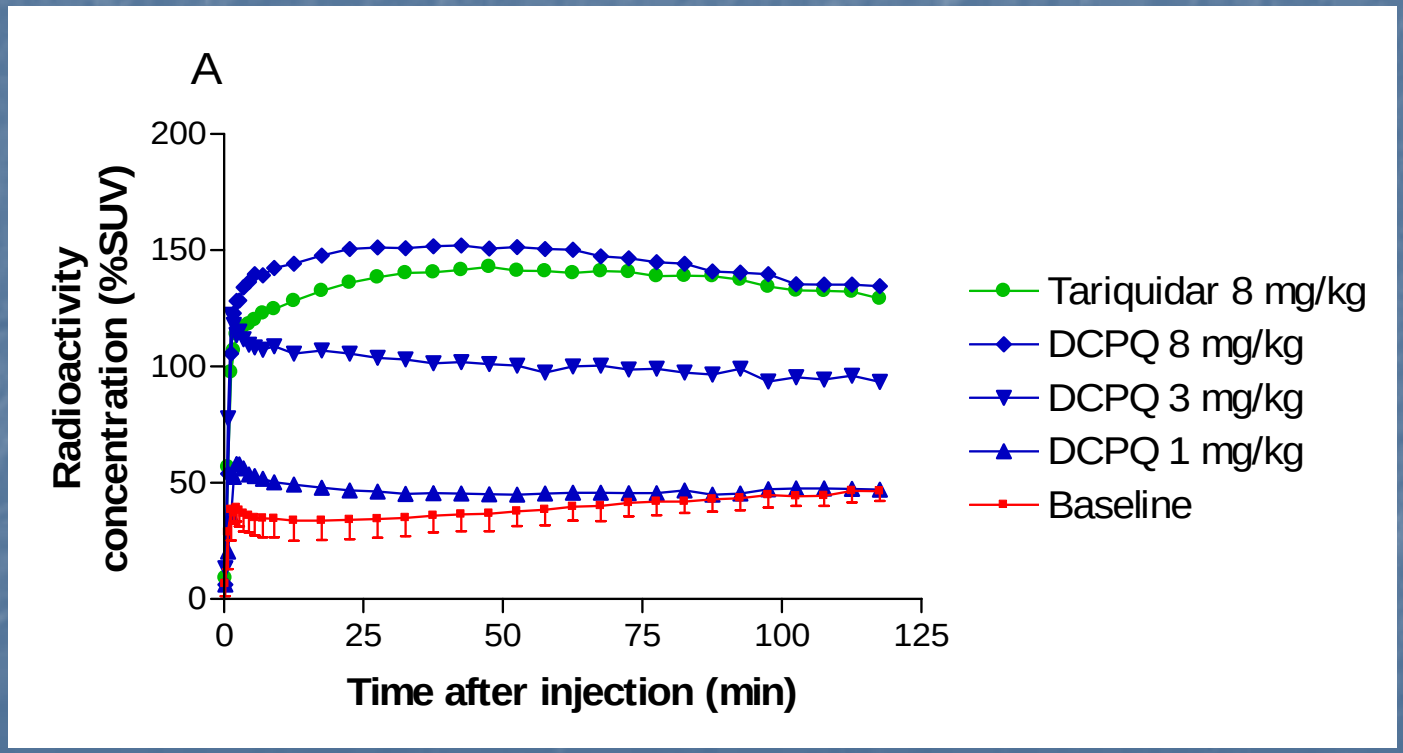
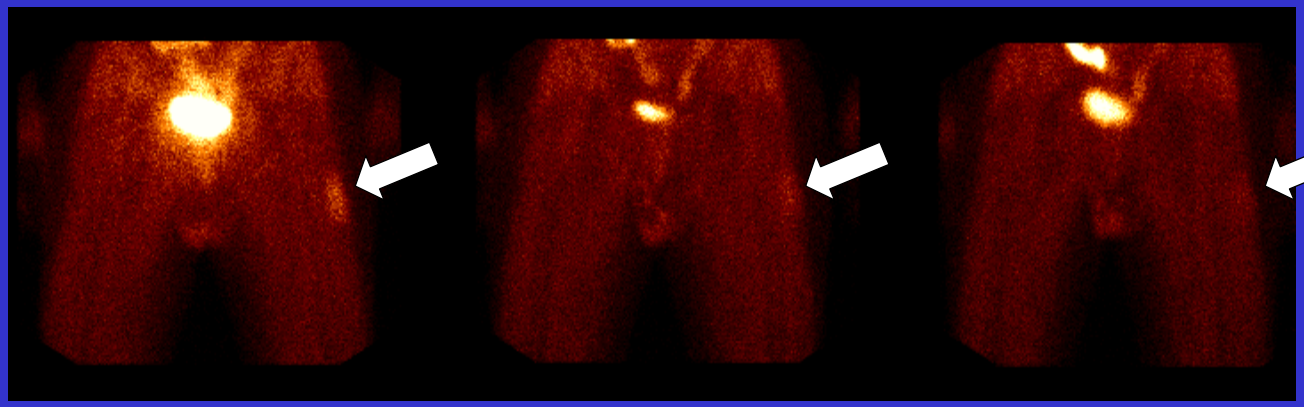


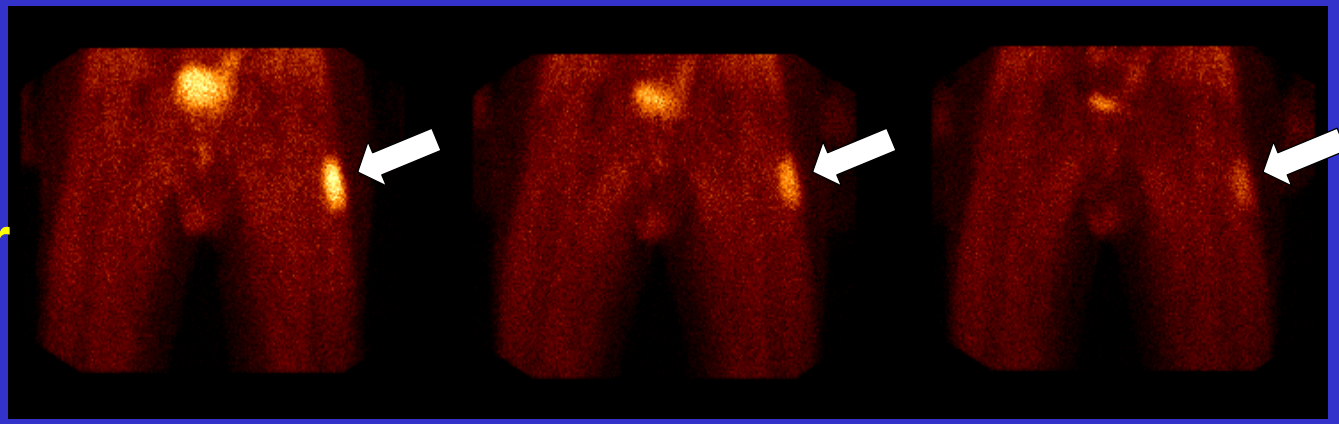
Renal Cell Carcinoma: Tariquidar increases uptake of ^{99m}Tc-Sestamibi in metastasis of thigh

Baseline



1 hour 2 hours 3 hours

After Tariquidar



Future Directions

1. BRAIN: Potential dysfunction of P-gp at blood-brain barrier: Alzheimer's disease, Parkinson's disease, epilepsy
2. ONCOLOGY: P-gp function in tumor cells transplanted into mice
3. Develop radiolabeled inhibitor to measure density, rather than function, of P-gp

Outline of Talk

1. PET: high sensitivity and specificity
2. Many PET ligands already exist to measure density of transporters – e.g., dopamine transporter in Parkinson disease
3. P-gp: efflux transporter “protects” organs like brain and testis from some toxins and drugs
4. [^{11}C]loperamide: avid P-gp substrate but has radiometabolite; measures function
5. [^{11}C]desmethyl-loperamide (dLop): metabolite is better than parent
6. After P-gp blockade, [^{11}C]dLop has high brain uptake that is dependent on flow
7. [^{11}C]dLop in humans: no brain uptake at baseline and slightly increased by P-blockade

ACKNOWLEDGMENTS

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Sami Zoghbi, PhD

Jeih-San Liow, PhD

Nick Seneca, PhD

OVERALL:

Director PET Radiochemistry: Victor Pike

Radiochemist: Neva Lazarova

Metabolism: Sami Zoghbi

Rodent Imaging & Image Analysis: Jeih-San Liow

Monkey Imaging: Robert Gladding

Human Imaging: Ferraris Araneta, NP; Chuck Kreisl, MD

Chemistry: Cheryl Morse, Jinsoo Hong, and Kelly Sprague

Self-Assessment Quiz: True or False?

- Loperamide, an antidiarrheal drug, lacks central nervous system opiate effects because P-gp (Permeability-glycoprotein) blocks its entry into brain.
- Positron emission tomography (PET) can measure the function of P-gp *in vivo* by using a radiolabeled P-gp substrate such as [^{11}C]loperamide.
- PET can monitor the *in vivo* metabolism of radioligands. By measuring P-gp function, PET can also monitor drug distribution.