PET Radioligands for Neuroscience and Drug Development

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Content

- Radioligand development
- Translational imaging
- PET for drug development

Today

PET at Karolinska Institutet – 1974 one of the worlds first PET- centers

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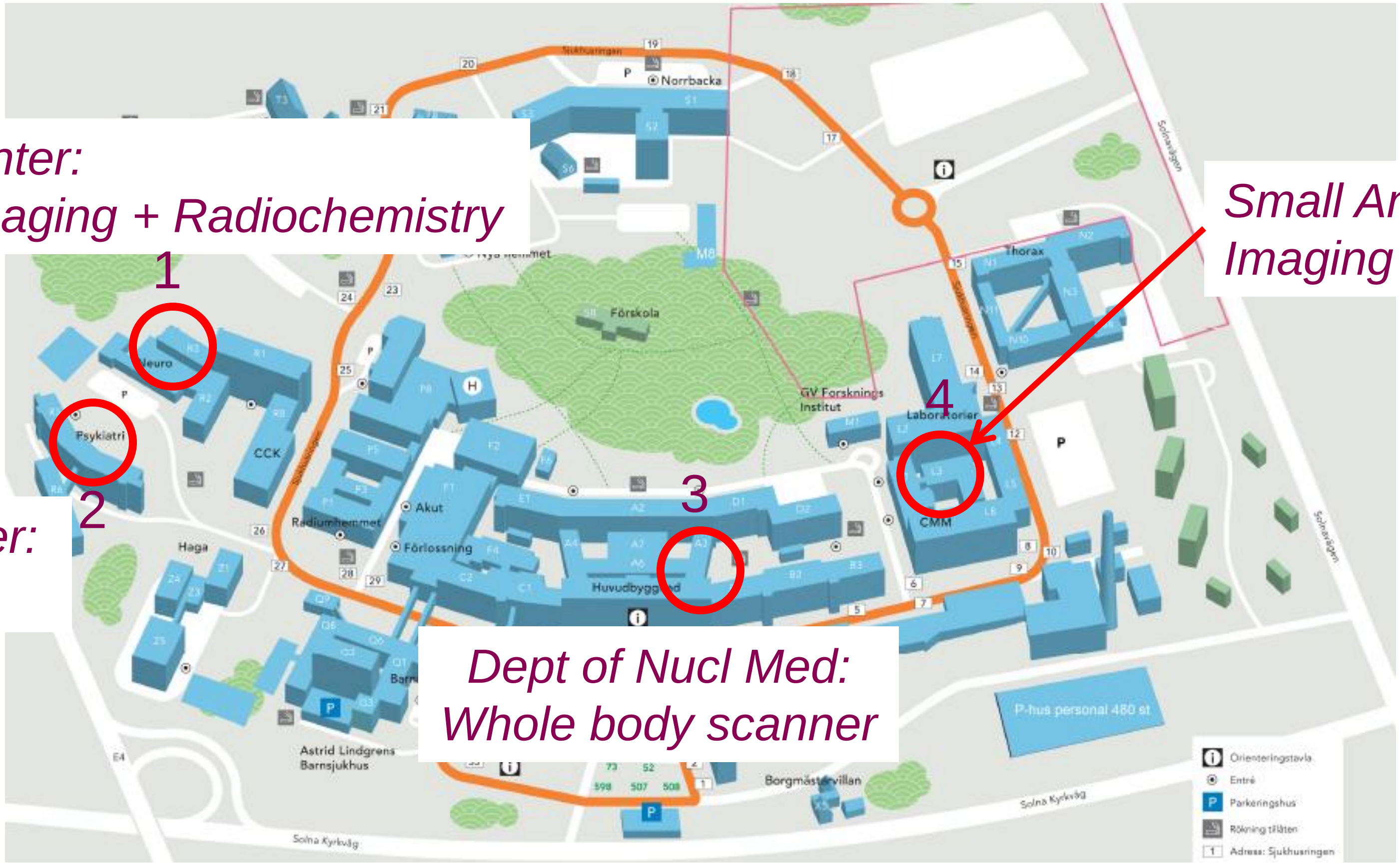

- Neuroimaging group of >70 employees
- Multidisciplinary: Physics, Radiochemistry, Biochemistry, Pharmacology, Biomathematics, Psychiatry, Neurology, Psychology

New Karolinska University Hospital (NKS)

2017

Future

NKS PET Centre 2017 – Beyond State-of-Art

Two cyclotrons, >30 hot-cells, GMP and preclinical hot-labs, human imaging and preclinical imaging

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”Main PET-research”

- Pure Academic 50%
  - Advancement of methodology
  - Basic neuroscience in man (brain function)
  - Clinical neuroscience
    - patophysiology, diagnostics

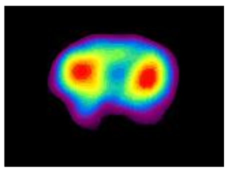
- Industrial - Academic 50% (70% budget)
  - collaborations on drug discovery and development

Novel Methods leading to
New Medications in Depression and Schizophrenia

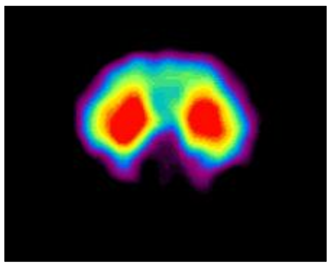
This study is part of the Innovative Medicines Initiative Joint Undertaking (IMI) under Grant Agreement N° 115008

Molecular Imaging now spans across translational from mouse-to-man

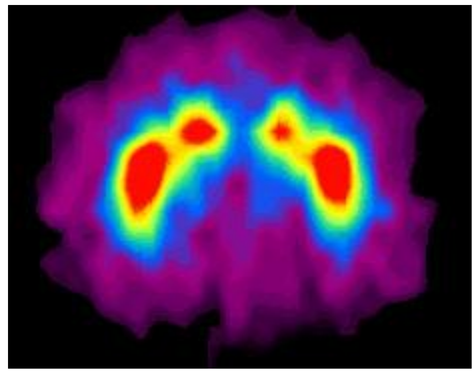
Mouse



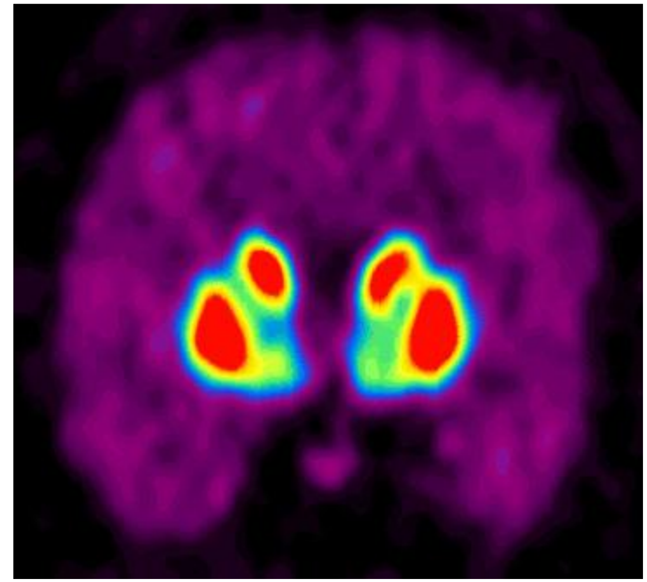
Rat

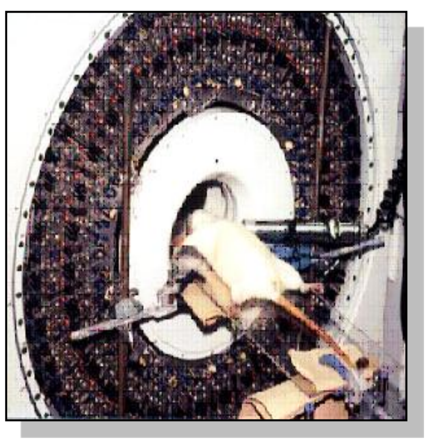


Monkey



Human





The druggable genome

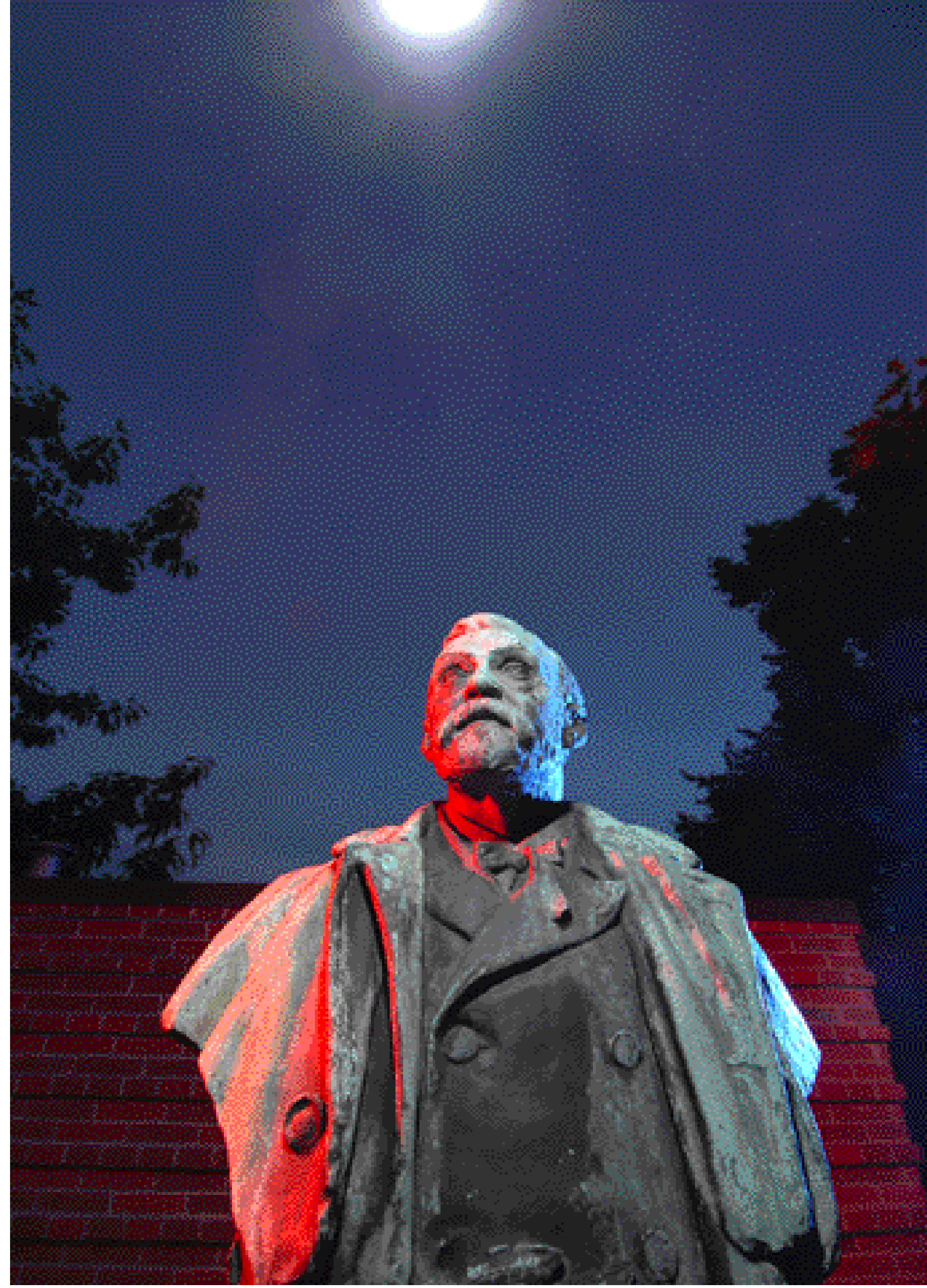
The genetic endowment

Human behaviour

Molecular Imaging is a
”Biochemical phenotype in vivo”

The NOBEL PRIZE

In Physiology or Medicine



Magnetic Resonance Imaging (MRI) 2003

Paul Lauterbur and Peter Mansfield

Comparison between different imaging modalities

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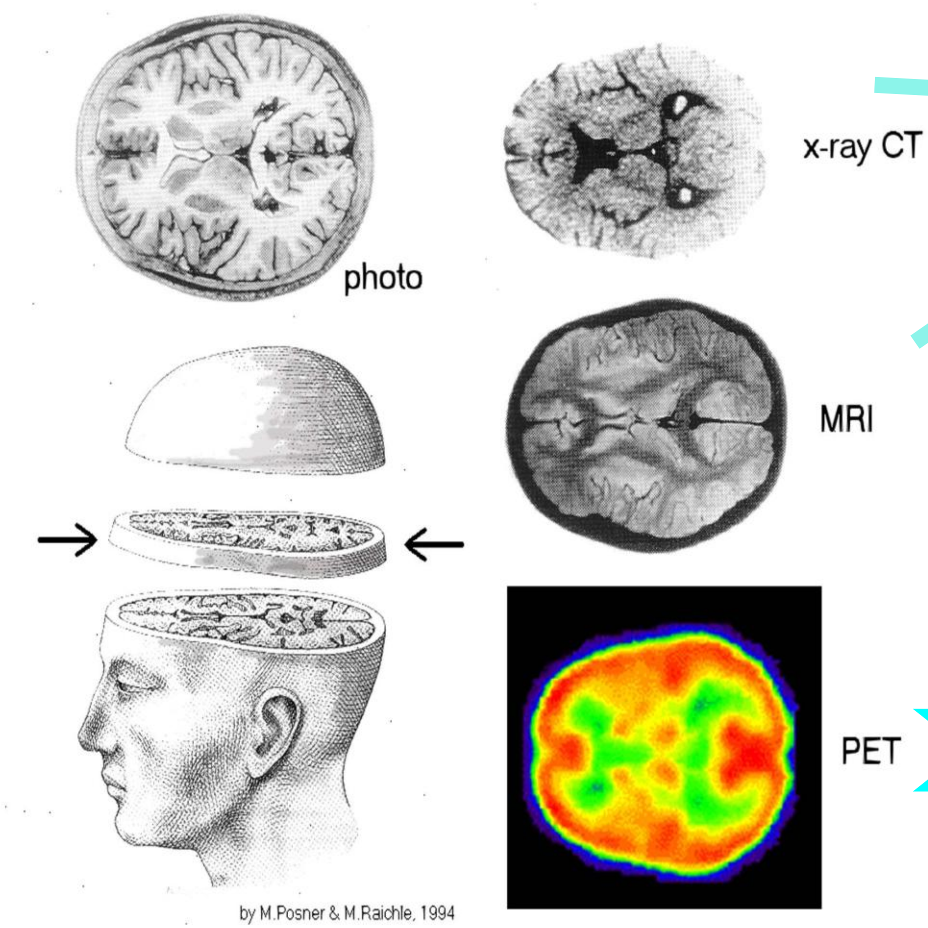
Modality	Spatial resolution	Depth	Temporal resolution	Sensitivity	Molecular probe
PET	1–2 mm	No limit	10 s-min	nmol-fmol	ng
SPECT	0.5–1.5 mm	No limit	min	pmol	ng
Bioluminescence	3–5 mm	1–2 mm	sec-min	fmol	µg-mg
Fluorescence	2–3 mm	<1 mm	sec-min	pmol-fmol	µg-mg
MRI	25–100 µm	No limit	min-hrs	mmol	mg
CT	50–200 µm	No limit	min	–	N/A
Ultrasound	50–500 µm	mm-cm	sec-min	–	µg-mg

One million times less mass

PET is Superior for Neuroscience and Drug Development

Examples of Tomography



X-ray Computed Tomography
Magnetic Resonance Imaging

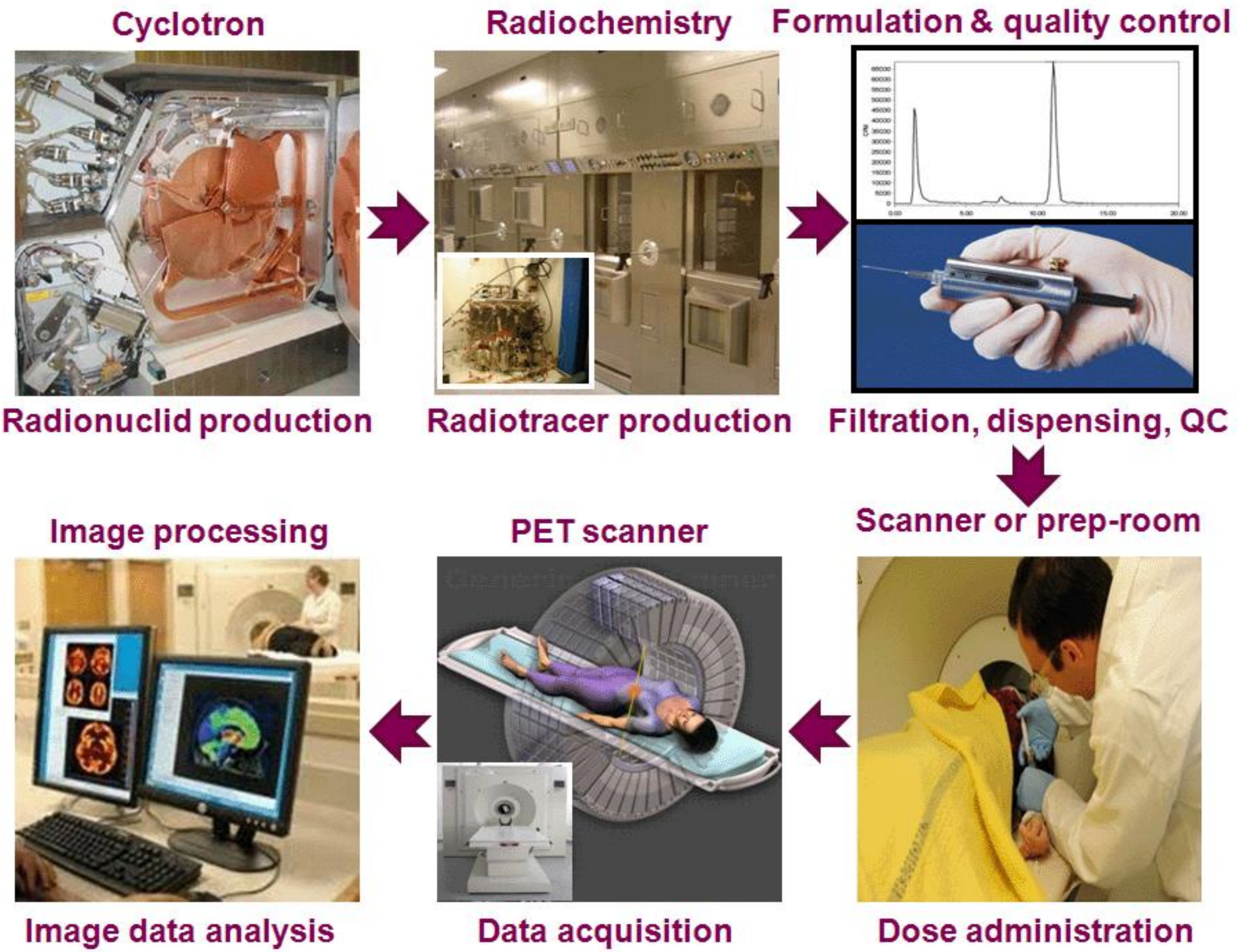
Imaging of anatomy

PET: imaging of functions:

- Blood perfusion
- Oxygen utilization
- Glucose consumption
- Aminoacid transport
- Receptors status

Uses radiotracers labeled with short-lived positron emitting isotopes

Infrastructure of PET



The radioligand challenge

- The human genome contains about 20.000 genes
  - Clamp et al, PNAS, 2007
- About 10 % of the proteins may serve as drug targets
  - Able to bind small molecules with appropriate properties
  - Half of them expressed in CNS
- > 400 targets have shown ’rule of five’ binding
  - of which 120 actually marketed, (Hopkins & Groom, 2002)
- 2017, about 40 proteins can be examined by PET in the human brain

The need for new PET radioligands is huge

Radioligand development at Karolinska Institutet 1982-2017

Monkeys
• 250 radioligands examined after radiolabelling

Man
• 45 radioligands examined
• 22 validated and suitable for applied studies world wide

- D2-dopamine
  - **[11C]raclopride (1985)**
  - [11C]FLB457 (1997)
- D1-dopamine
  - [11C]SCH23390 (1985)
  - [11C]NNC112 (1995)
- Dopamine transporter
  - [11C]PE2I (2004)
  - **[18F]FEPE2I (2009)**
- Serotonin transporter
  - [11C]MADAM (2003)
- Serotonin 5HT2a
  - [11C]MDL100907 (1997)
- Benzodiazepine
  - [11C]Flumazenil (1986)
  - [18F]Flumazenil (2006)
- Norepinephrine transporter
  - [18F]FD2MeNER (2006)
- Serotonin 5HT1B
  - [11C]AZ10419369 (2008)
- Amyloid Plaque
  - [11C]AZD2184 (2008)
  - **[18F]AZD4694 (2009)**

Our aim should be to speed up the development of:

- new radioligands
- imaging biomarkers of pathophysiology

Why using fluorine-18?

^{18}F has longer half-life than ^{11}C, 110 min vs 20 min

Radioligands can be shipped for use at centers not having a cyclotron

Commercial potential as a radiodiagnostics

Why using carbon-11?

All organic compounds contain carbon

Large utility

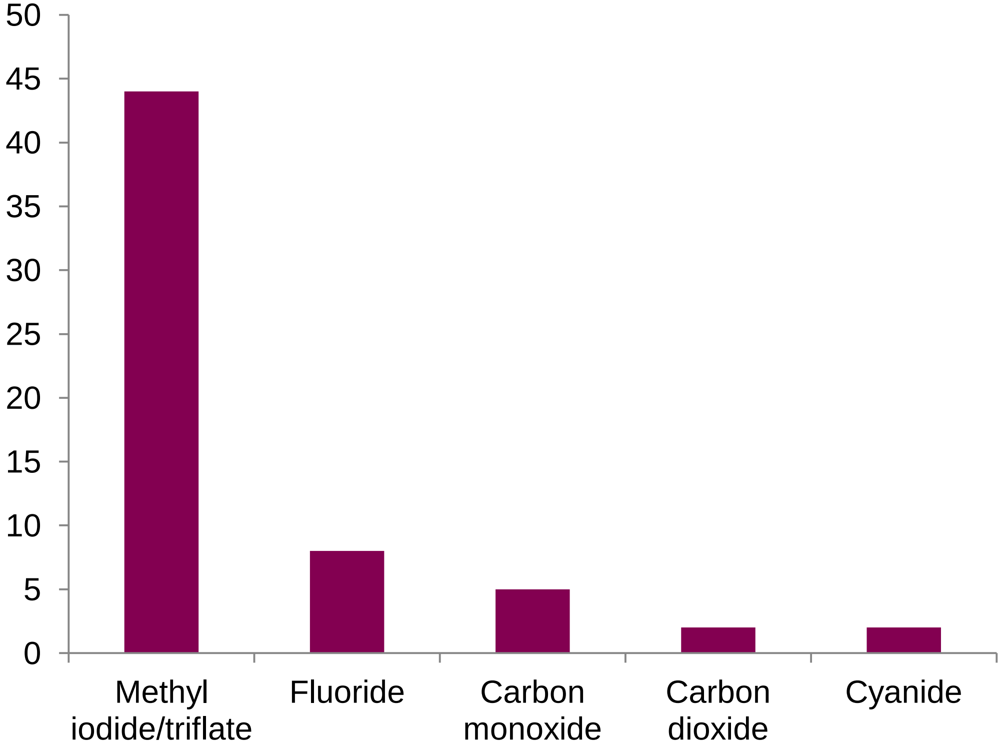
+ ^{11}C replaces ^{12}C without altering phys chem or pharmacological properties
+ Most drugs or druglike compounds will be possible to label with ^{11}C

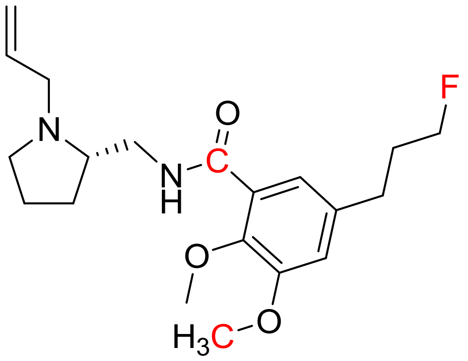
Short half life

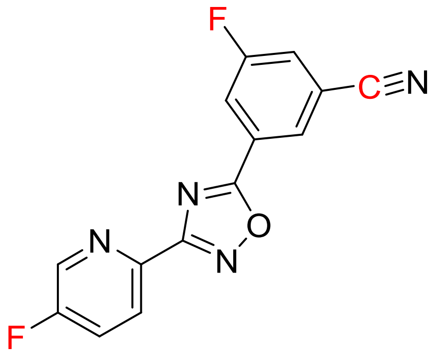
- Time constraints on radiochemistry
+ Favorable dosimetry
+ Low carry-over of radioactivity

} suitable for multitracer protocols

Radiolabeled compounds in the AZ/Karolinska PET alliance 2006-2015



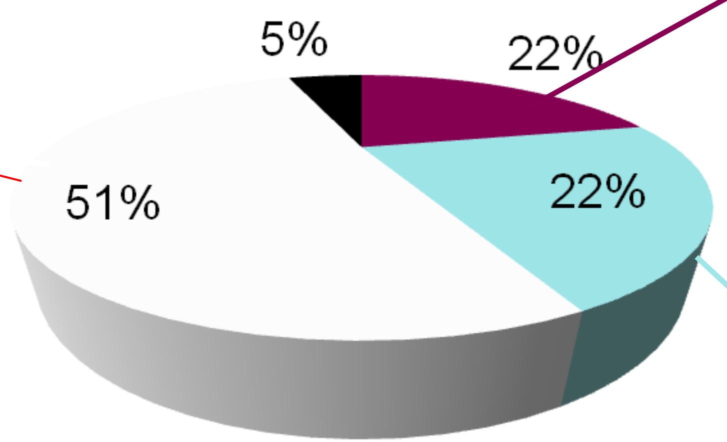




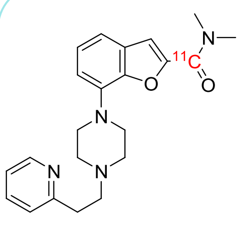
More than 70% still labeled via methylation

Diverse radiochemistry provides access to radiolabeled druglike molecules for PET

FDA approved NMEs in 2014 divided into labeling technique

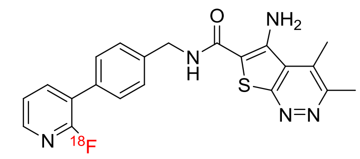


Fluorination　Methylation
Carbonylation　NA



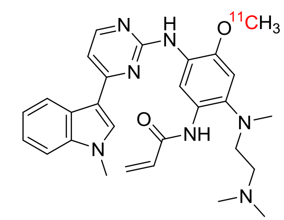
AZD3676

Carbonylation
Typical turnover:
6-12 weeks



AZ13713967

Fluorination
Typical turnover:
4-8 weeks



AZD9291

Methylation
Typical turnover:
2-4 weeks

New Molecular Entity (NME)

Improvements in technology provides efficient radiochemistry

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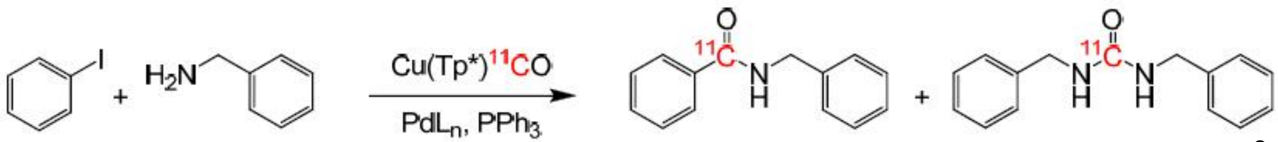
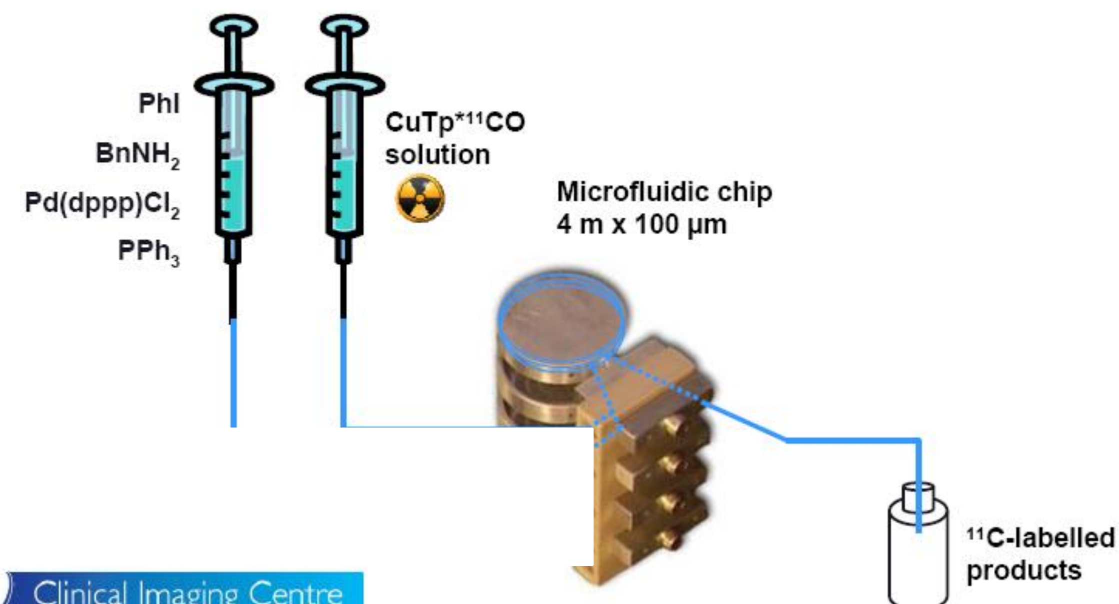
Radiochemistry Challenge

- Microfluidic system installed 2010 and….



- …combined with a CO- system..



- … provides capacity to label more compounds requiring complex radiochemistry

PET Radioligand Criteria

- ***Chemistry***
  - LogP 1.5-3
  - Radionuclides: ^{11}C, ^{18}F
  - Introduce label late in the synthesis (high SR)
- ***Biochemistry***
  - Low non-specific binding
  - Affinity: nanomolar (@ 37^{0} C)
  - Reversible binding (straight-forward compartment analysis)
- ***DMPK***
  - Favourable brain disposition (Low Vd, high free plasma fraction)
  - Not a PGP-substrate
  - Radioactive metabolites should not pass BBB
  - Rapid metabolism not necessarily a disadvantage

An ideal PET tracer for receptor binding

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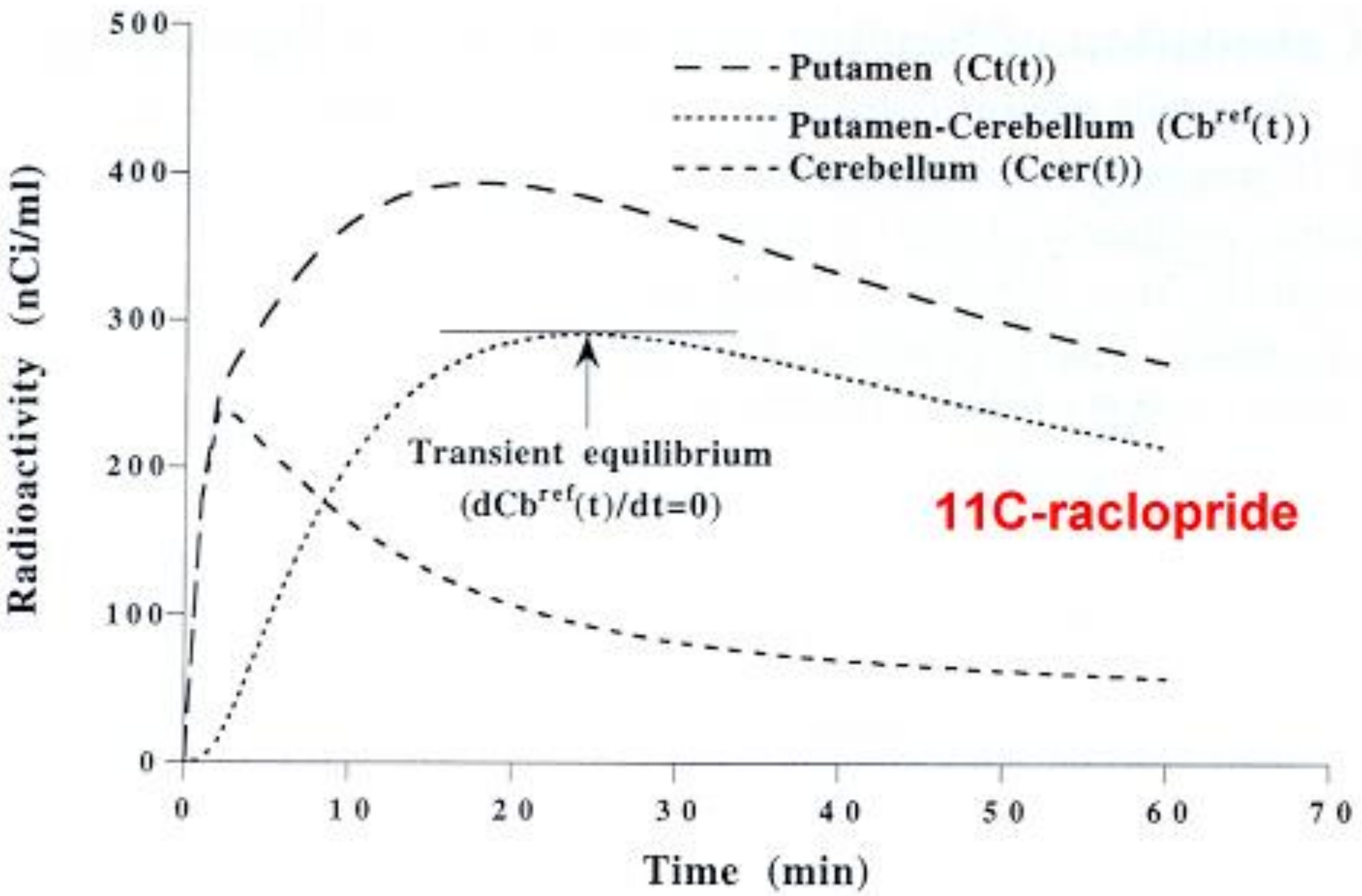
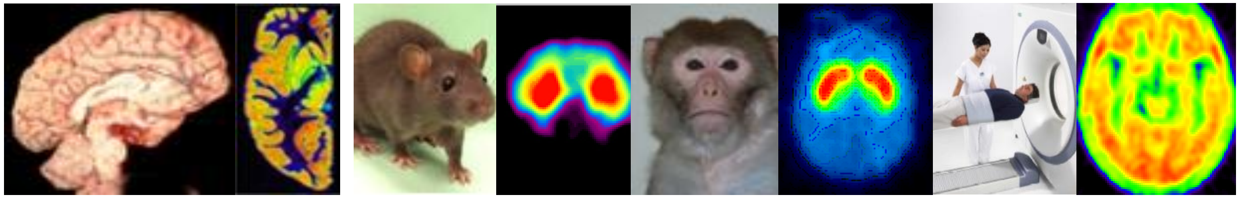


Farde et al Science 1986

A complete translational molecular imaging platform

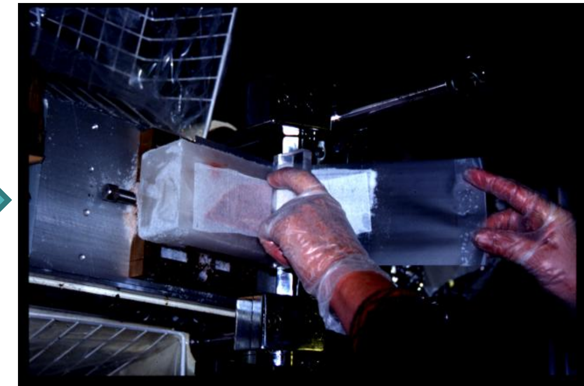
Ex vivo	In vivo		
	Back-translation ←		→ Translation
Post mortem brain studies: rodent, NHP, human brain	Rodents (rats, mice; wildtype, transgenic)	Non-human primates (NHP)	Human subjects: volunteers, CNS patients

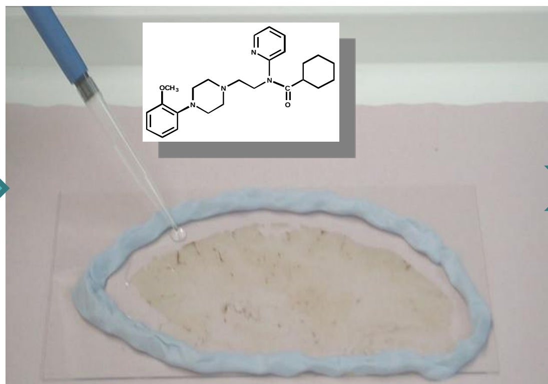


Autoradiography, immunohisto-chemistry, neuro-blot, histoblot, ...	μPET	NHP PET	Human PET
	animal disease models, ...	microdosing, occupancy, distribution, dosimetry, bioavailability, ...	


Brain collection


Embedding

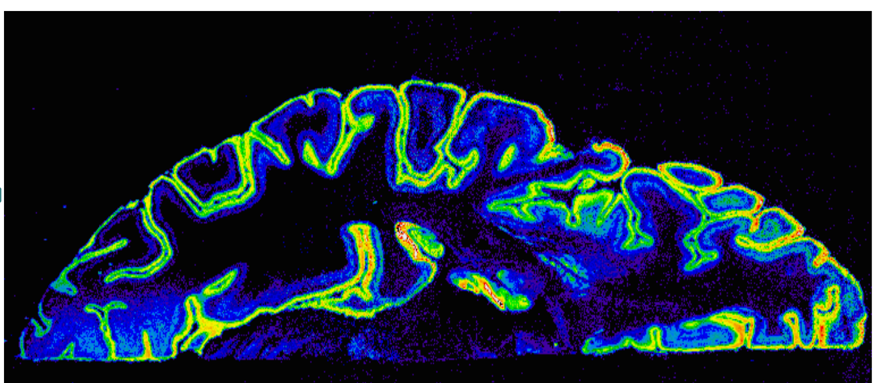

Brain sectioning



Incubation


Exposure


Image development


Visualisation

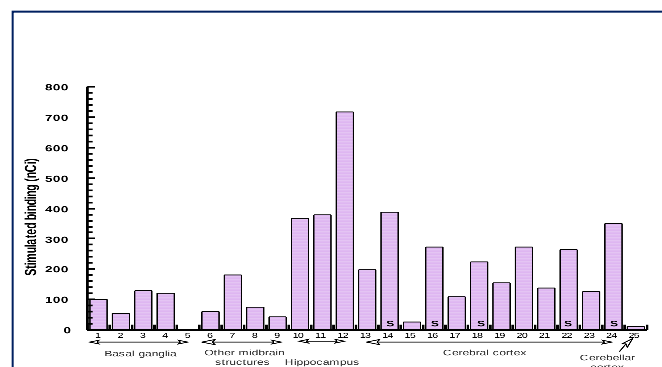
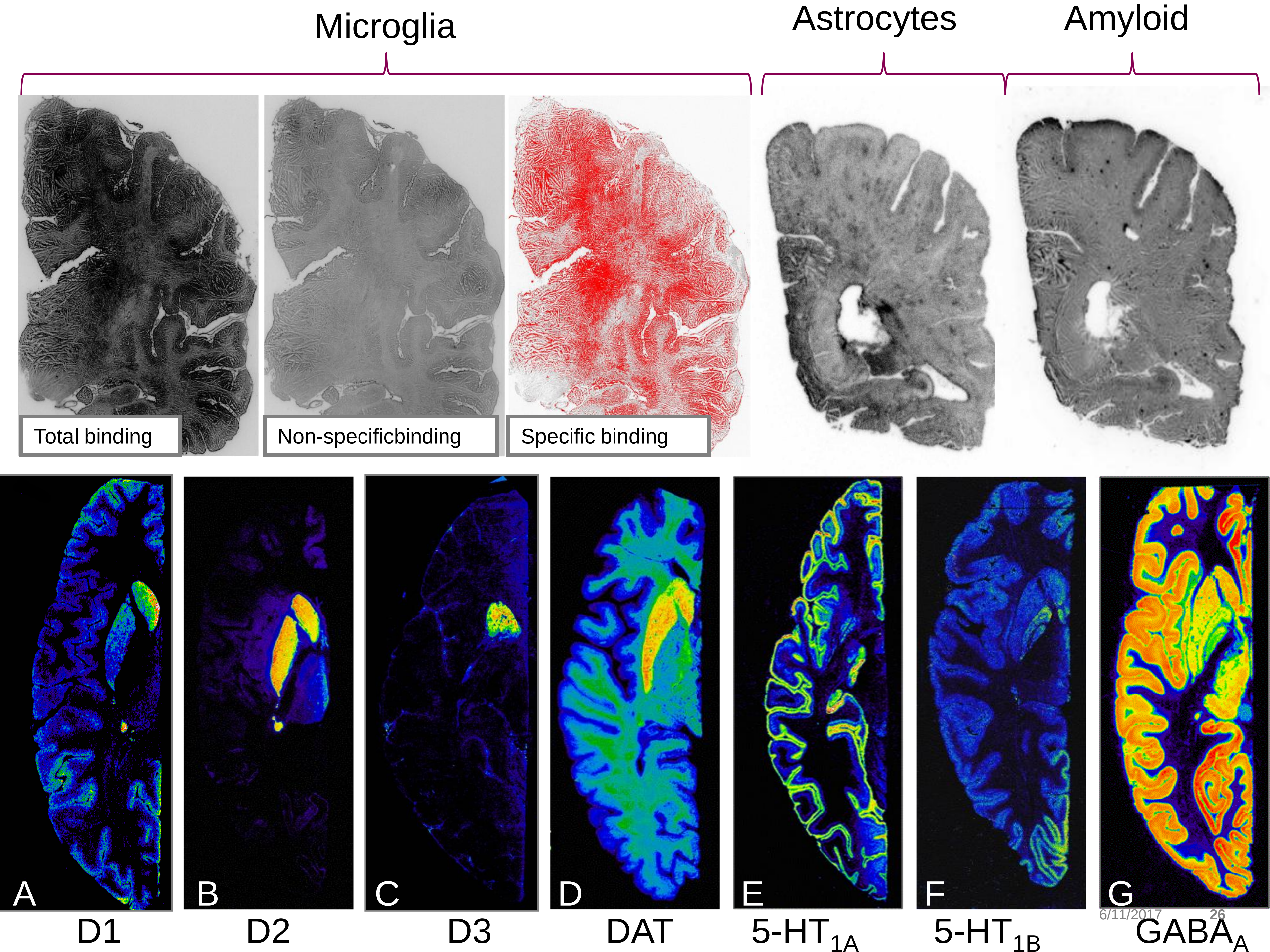


Image quantification



Microglia
Astrocytes
Amyloid
Total binding
Non-specificbinding
Specific binding
A
B
C
D
E
F
G
D1
D2
D3
DAT
5-HT1A
5-HT1B
GABAA

Why small animal?

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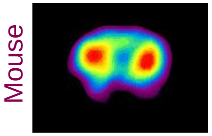
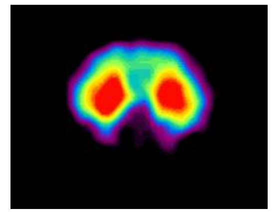
Species difference

Requirement for
high radioactivity,
high specific activity
in extremely small volumes

Mouse



Rat

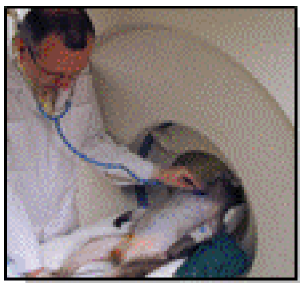


Small animal disease models:
Transgene, toxin, etc.

Longitudinell studies

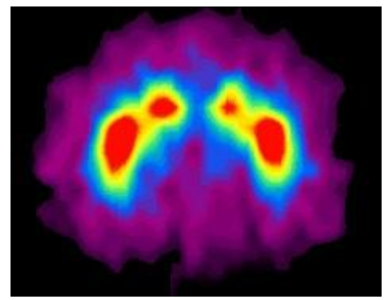
Back-translational validation

Molecular imaging biomarkers



Cost

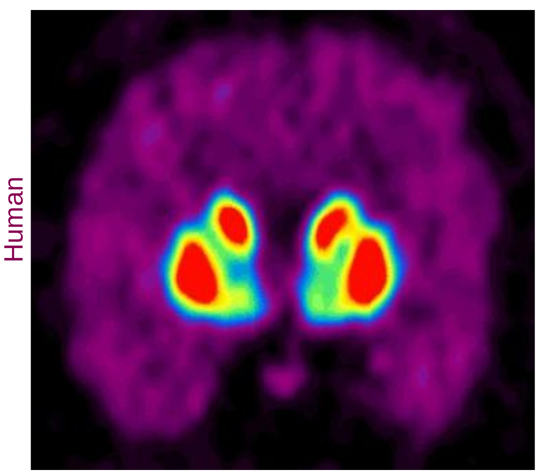
Monkey



Primate

Restrictions
(ethical,
radiation safety, MPA)

Human



Direct observations

Disadvantages

Advantages

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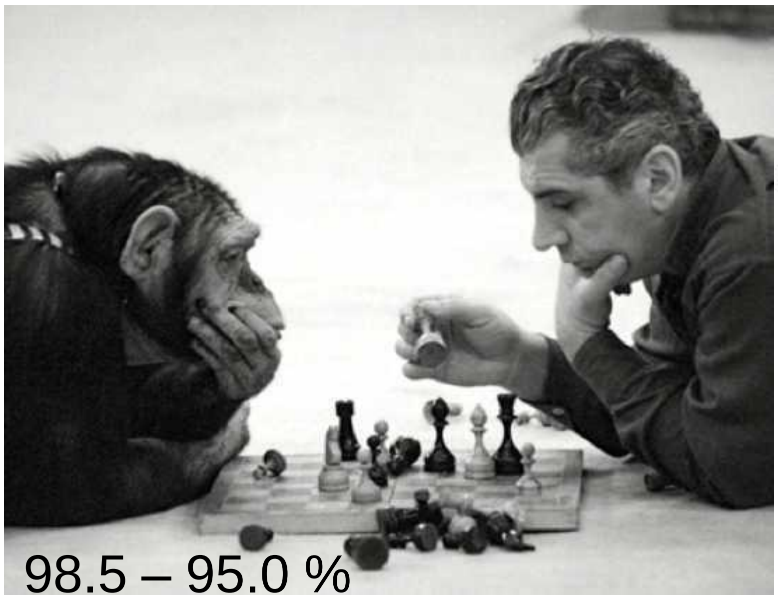
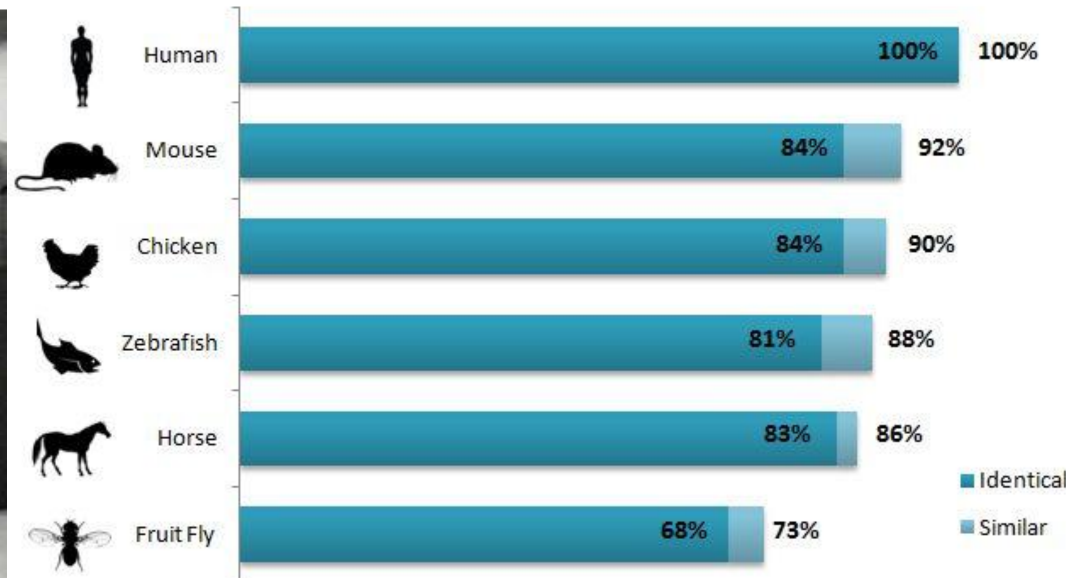
DNA Homology:

How closely are two or more separate, but related, strands of DNA to each other, based on their base sequences.





P-gp Protein[a]	% of Amino Acid Homology[b]						
	Human	Chimpanzee	Rhesus Monkey	Dog	Guinea Pig[c]	Rat	Mouse
Mouse	87	80	85	83	82	93	100
Rat	85	83	88	83	80	100	
Guinea pig[c]	82	82	82	82	100		
Dog	87	89	87	100			
Rhesus monkey	93	94	100				
Chimpanzee	97	100					
Human	100						

Britten, PNAS, 2003

Syvanen et al., 2003

The scanner challenge – rodent - high resolution & sensitivity

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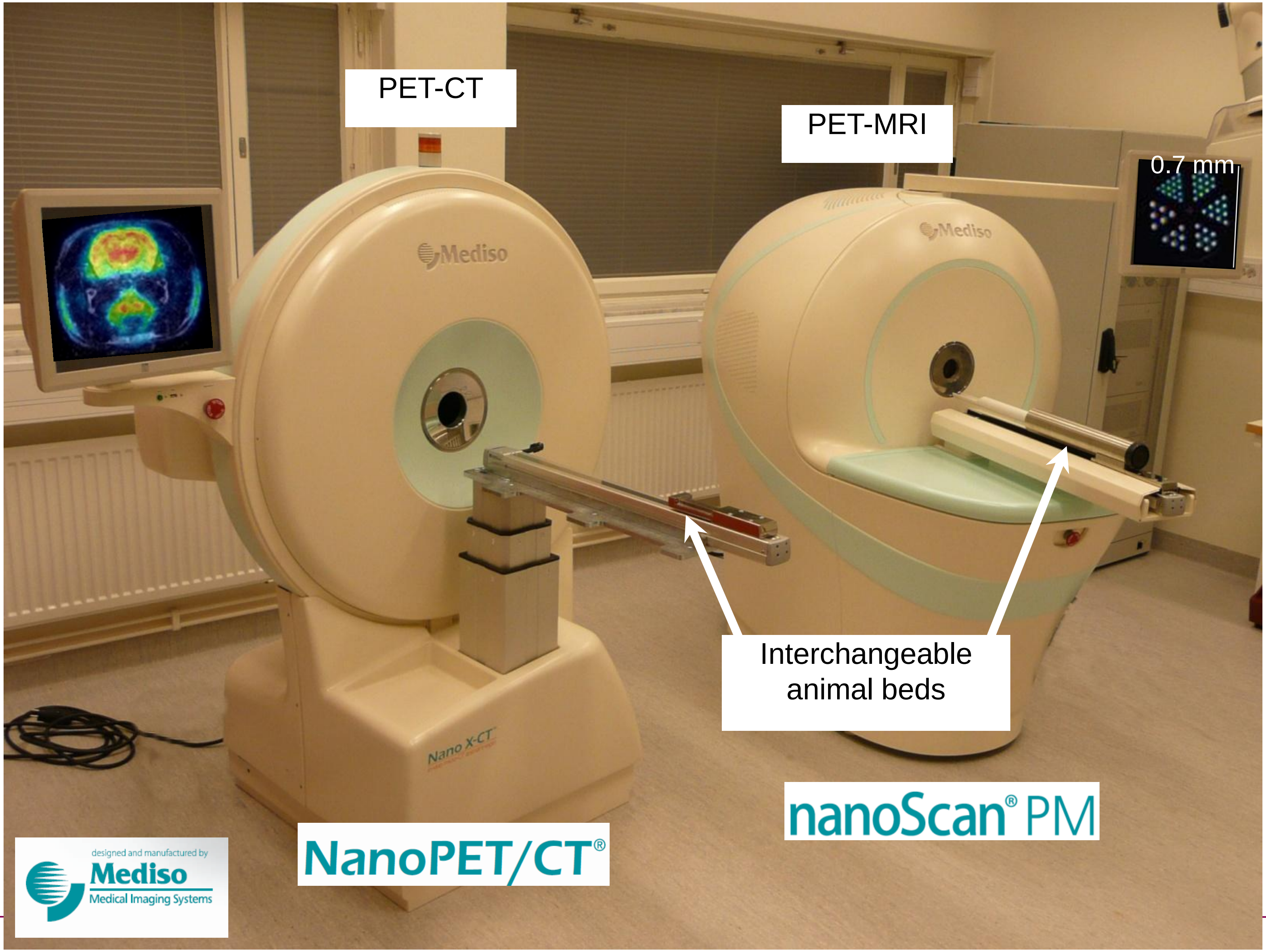
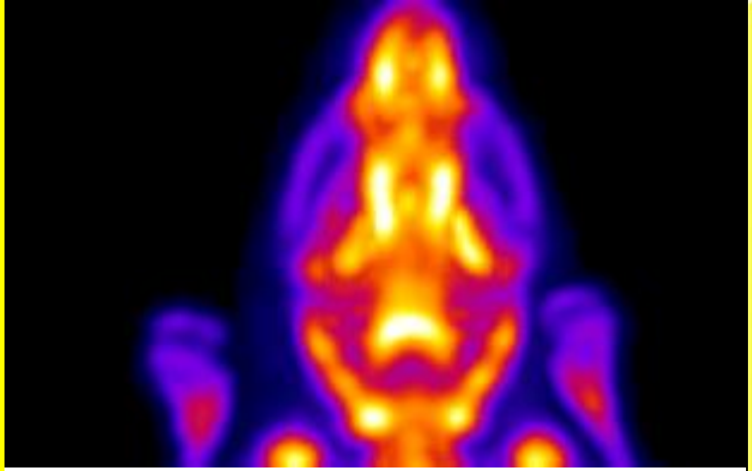
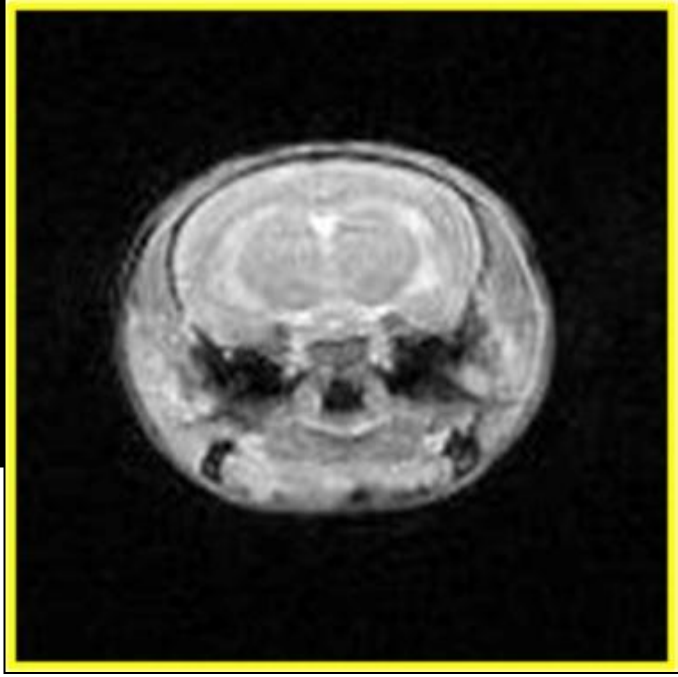




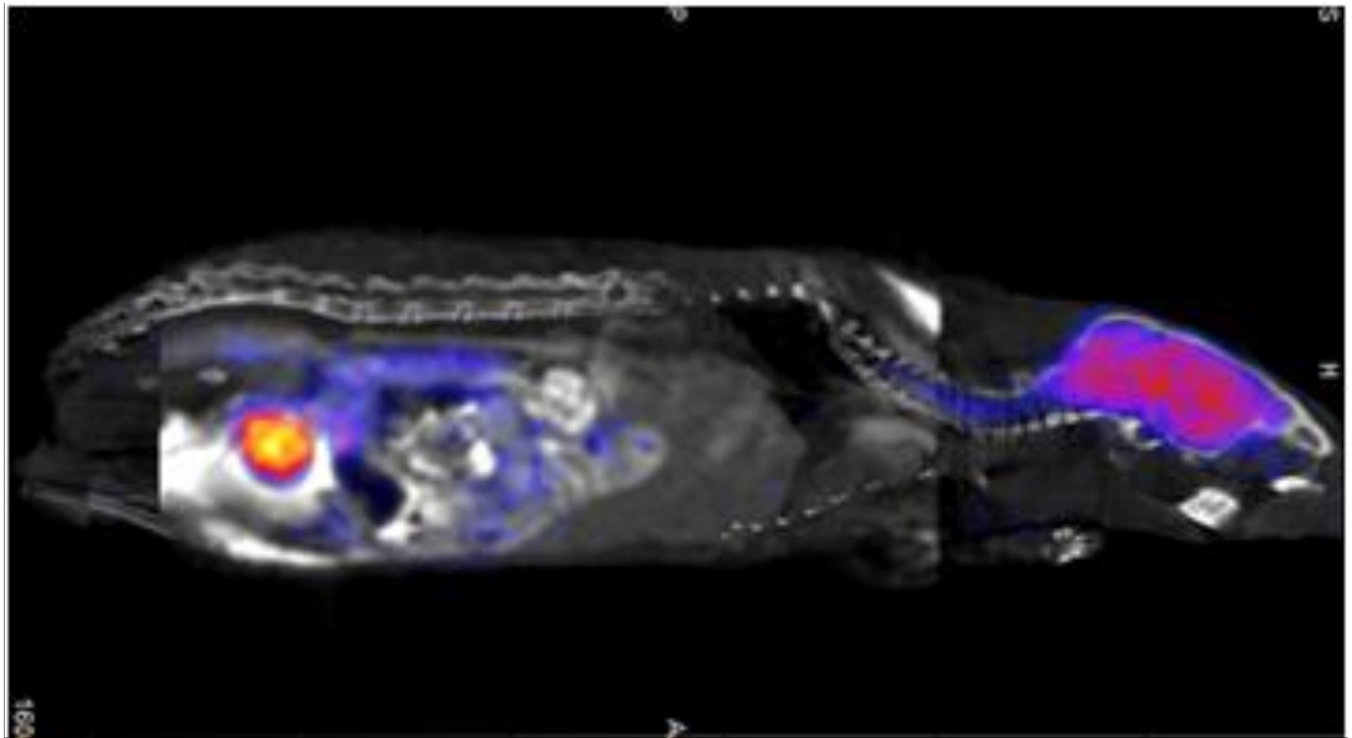
PET CT MRI



Bone-soft tissue contrast

Biochemical information

Soft tissue compartments; grey matter-white matter contrast



100 µm (first standard magnet MRI)

700 µm (world-best)

10 µm (world-best)

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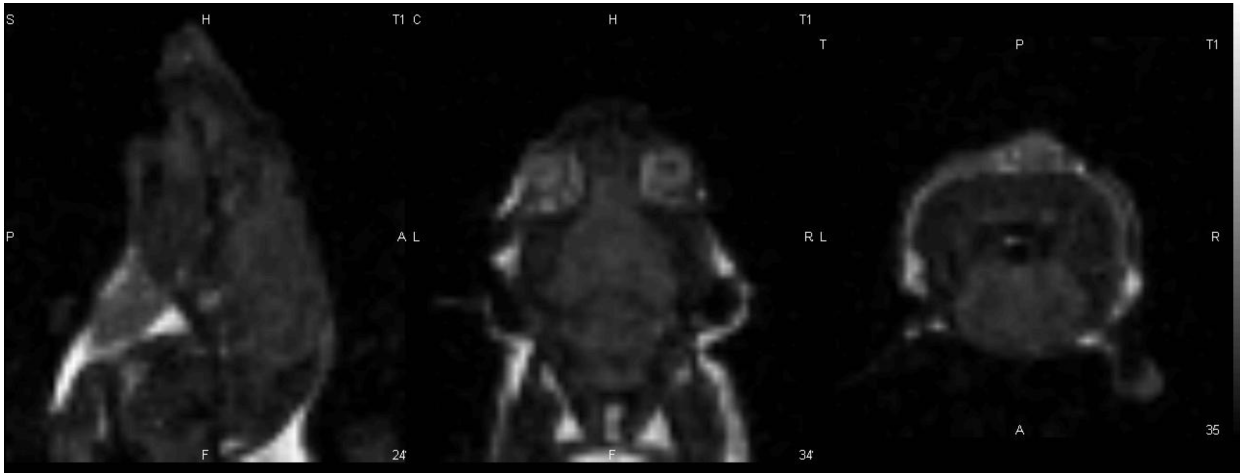
Dopamine D2

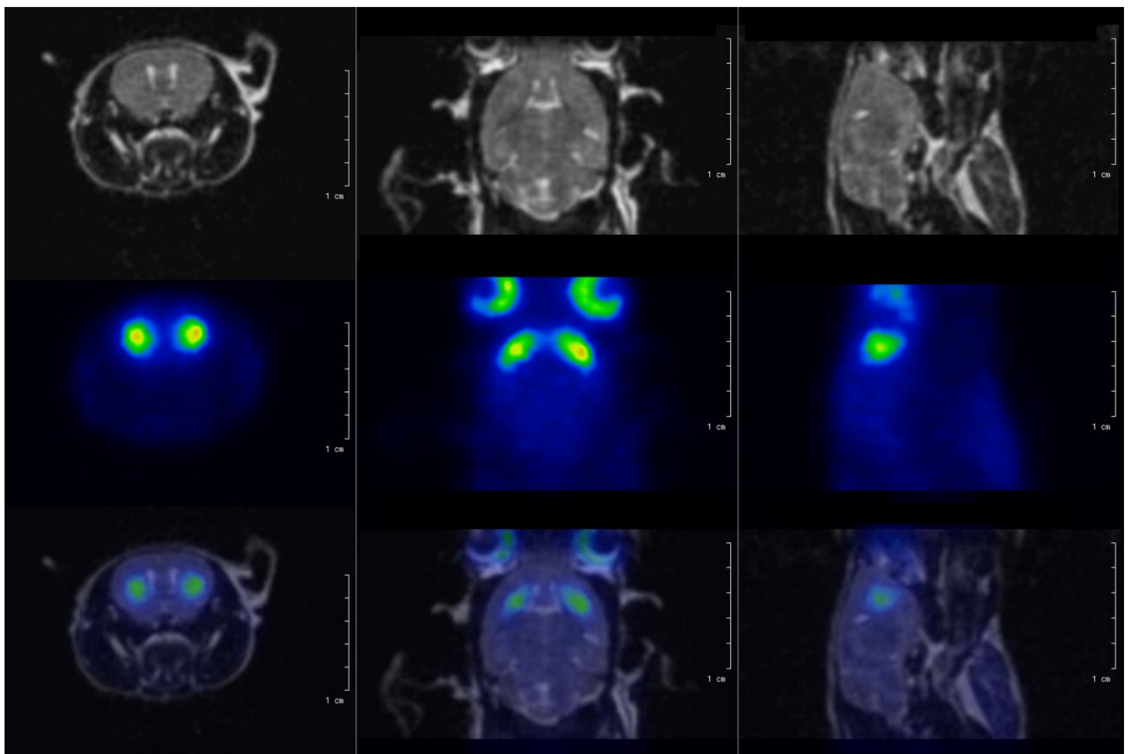


^{11}C-Raclopride (28.3 g male mouse, 13 MBq, 63 min scan)

MRI

PET

MRI + PET



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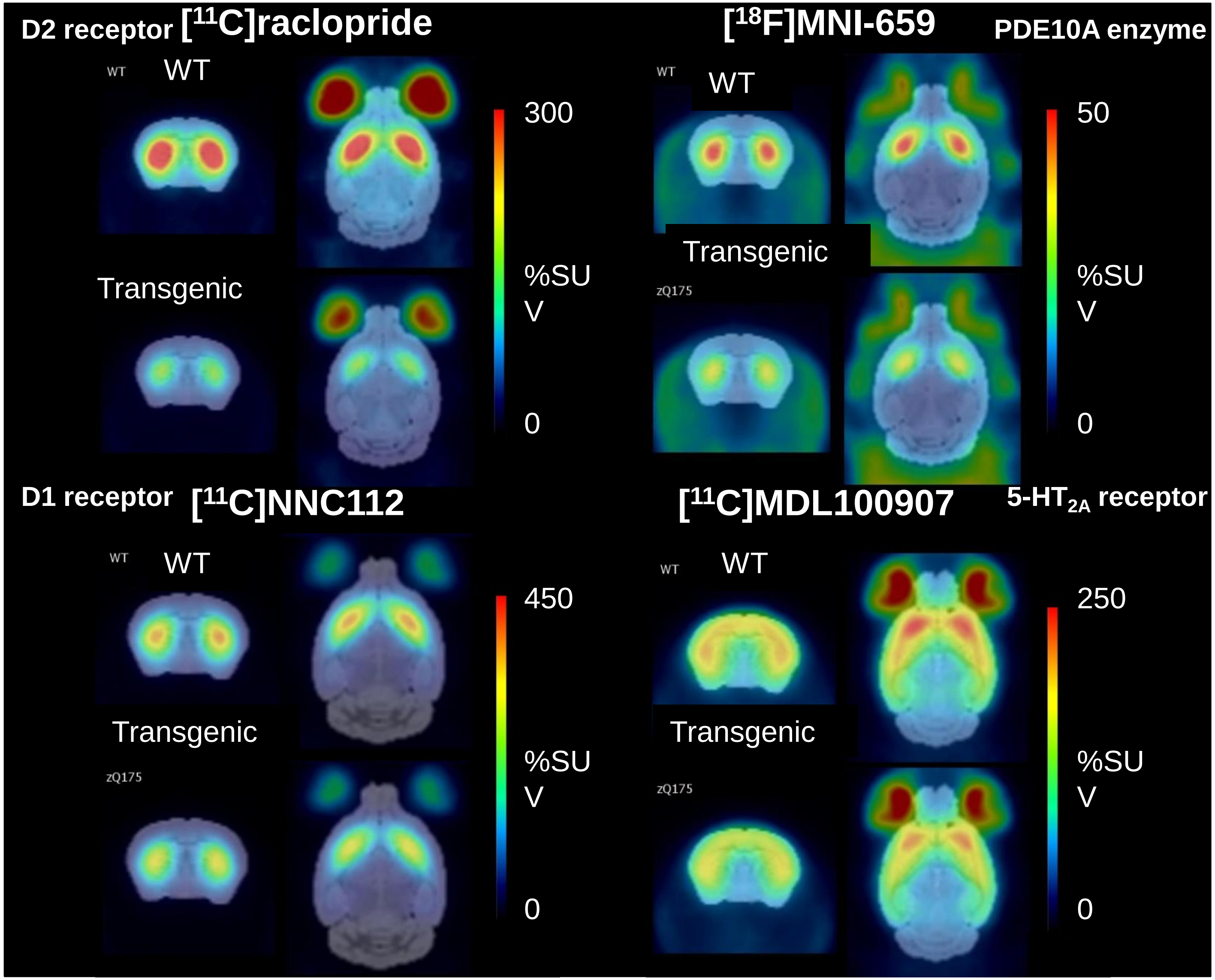
”Humanised” small animal disease models

Genetic	Biota	Infection	Chemical	Traumaic	Inflammatory
Knock out	Germ free	HVE	6-OHDA	Percussion	Inflammatory Bowel Disease Mouse
Knock in	Specific Pathogen Free	Dengue	DSS	Cooling	
Transgenic		Malaria	TNBS	Occlusion	

Animal model for Huntington's disease. WT and transgenic mice



Why is PET a ”hot” methodology in CNS drug discovery development?

Several major applications

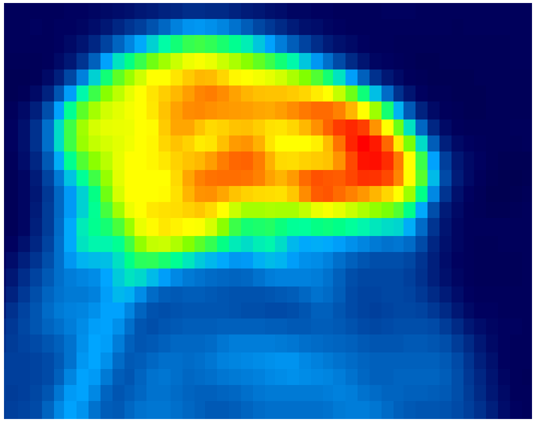
- Microdosing
- Drug occupancy at target of interest
- Imaging biomarkers of pathophysiology

Brain exposure is critical for CNS-drugs

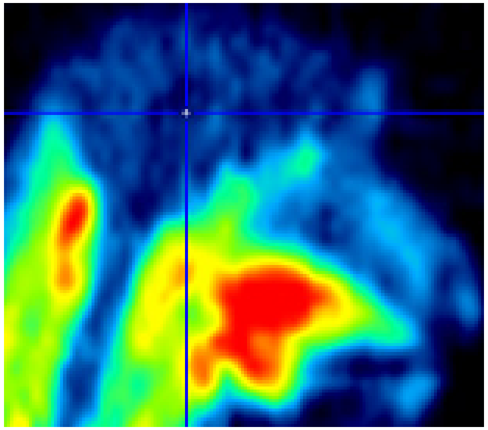
→ Low brain exposure is a significant reason for failure Taylor E.M. (2002) Clin. Pharmacokinet. 41:81-92.

→ Can be controled for by PET-microdosing

- Injection IV of less than 1 microgram of labeled drug

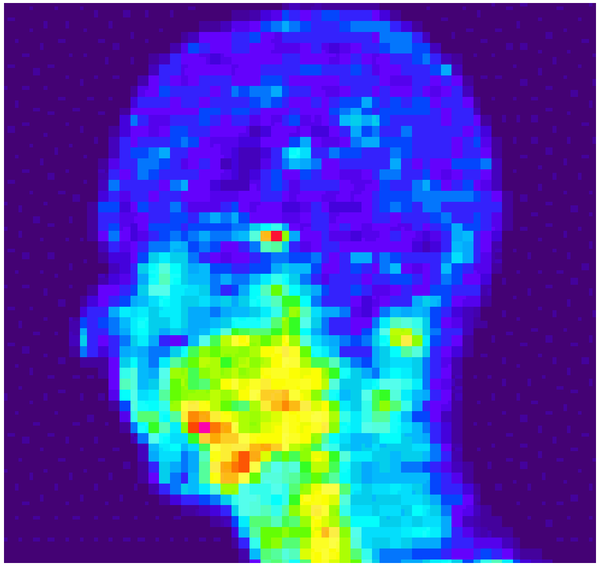


- Primate study
- CNS drug
- High brain exposure



- Primate study
- Drug for targets outside brain
- Low brain exposure

- PET microdosing
  - → Radiolabeling with C-11 (half-life 20 min)
  - → Measures drug concentration in target organ
  - → < 1 μg total, small tox-package, also pre-CD



Human study
Failing CNS-drug

Why is PET a ”hot” methodology in CNS drug discovery development?

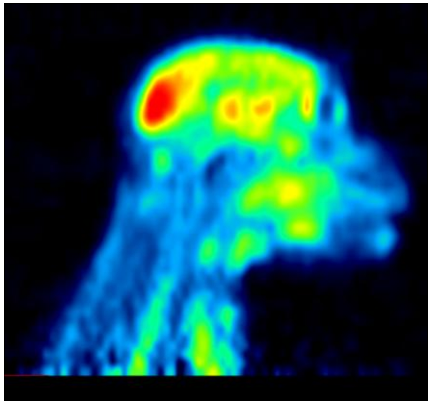
Several major applications

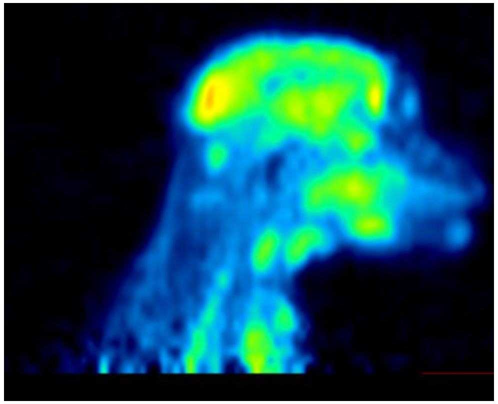
- Microdosing
- Drug occupancy at target of interest
- Imaging biomarkers of pathophysiology

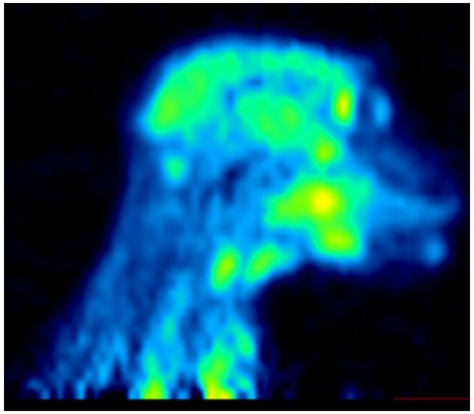
Receptor occupancy

Will often require development of a new radioligand

- Measures drug binding to a target protein (PoM)
- Applied for dose-finding, dosage regimes, and may serve as surrogate endpoint (PoC).


Baseline


0.25 mg/kg

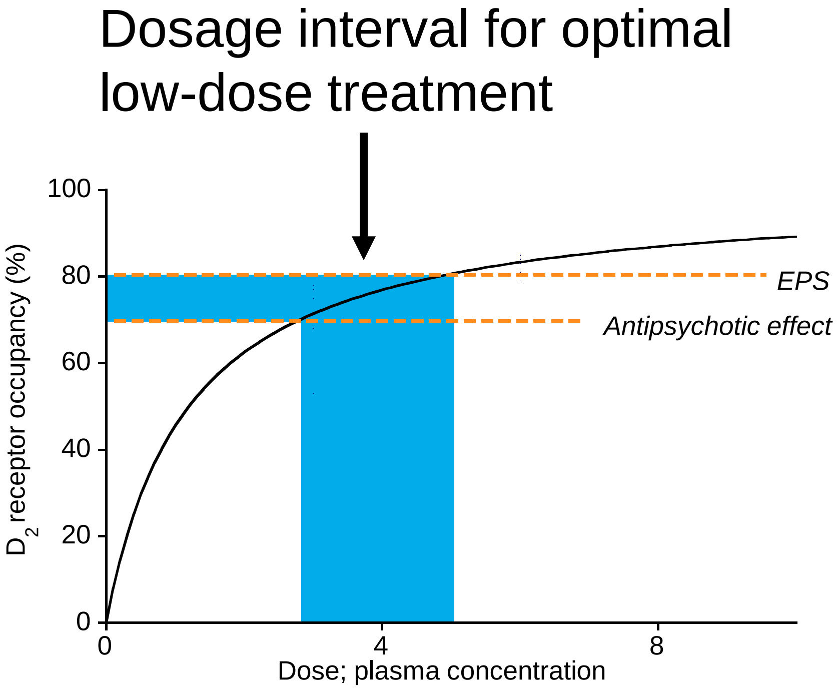

2.0 mg/kg

- Relationship between dose, plasma conc and occupancy in primates
  - Validity of dose range tested in man

The perfect dose?

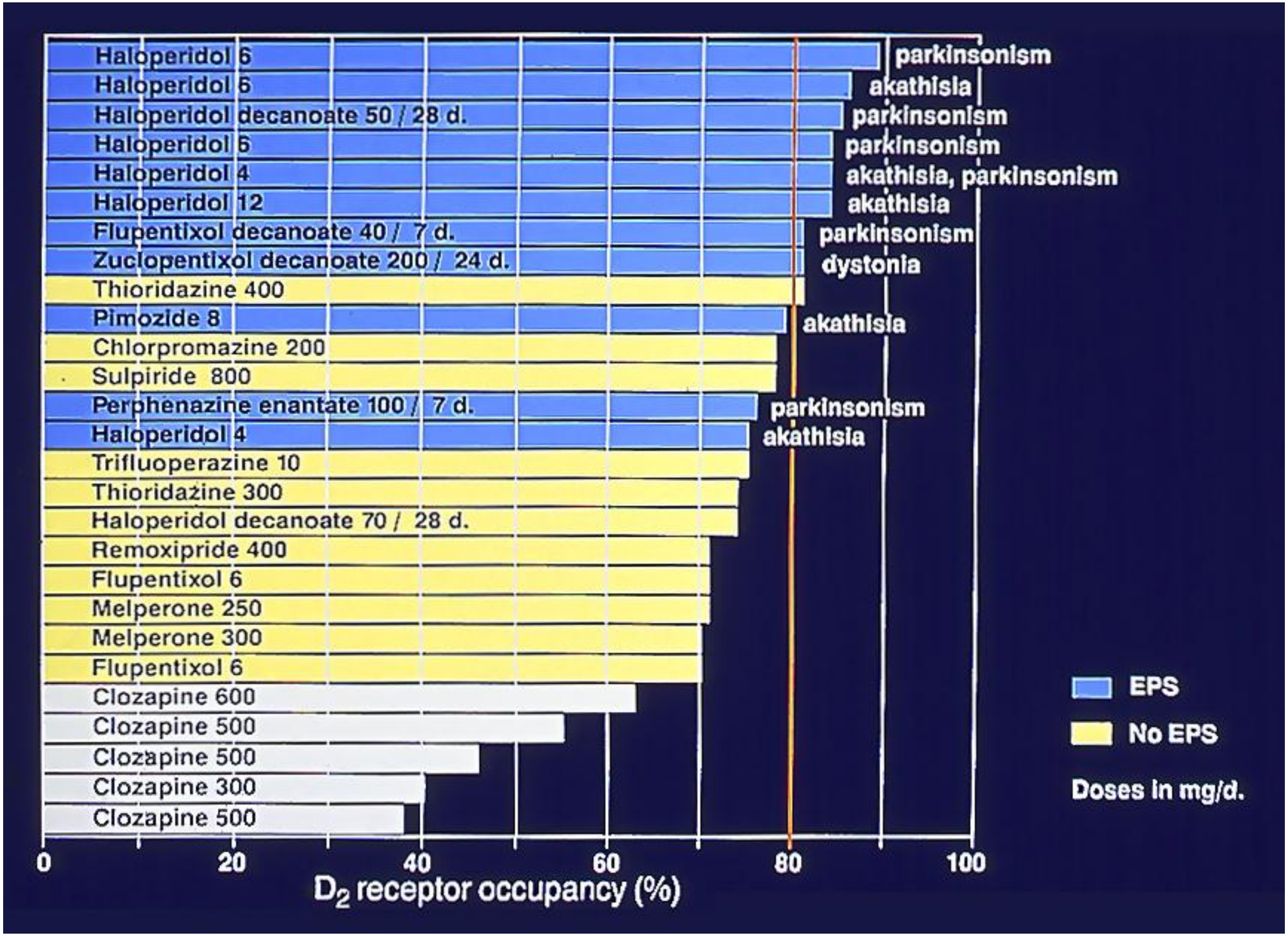
Improved daily life – a benefit to patients

Imaging studies with [11C]raclopride provided new guidelines for antipsychotic drug treatment of schizophrenia



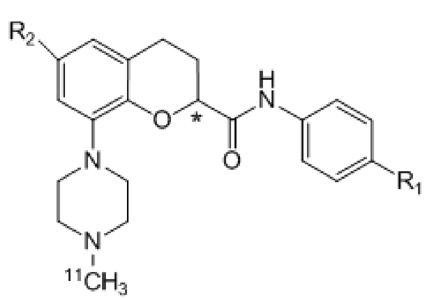
Farde et al, *Arch gen Psychiatry,* 1992

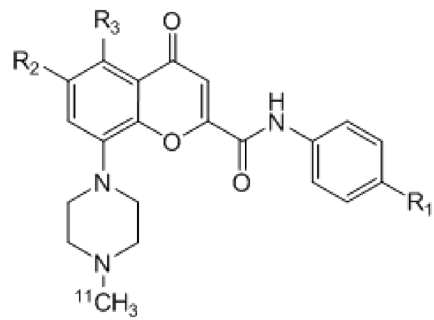
D_2 receptor occupancy induced by clinical doses of antipsychotic drugs

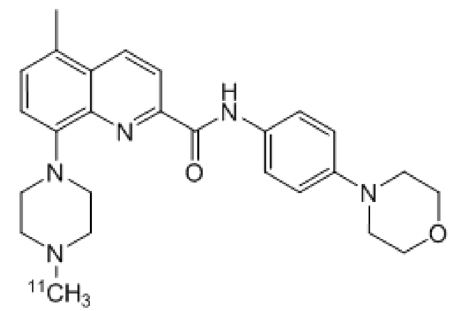


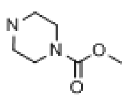
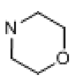
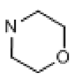
Farde et al, 1989,1992

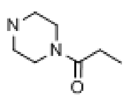
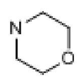
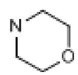
Development program 5-HT_{1B} radioligand





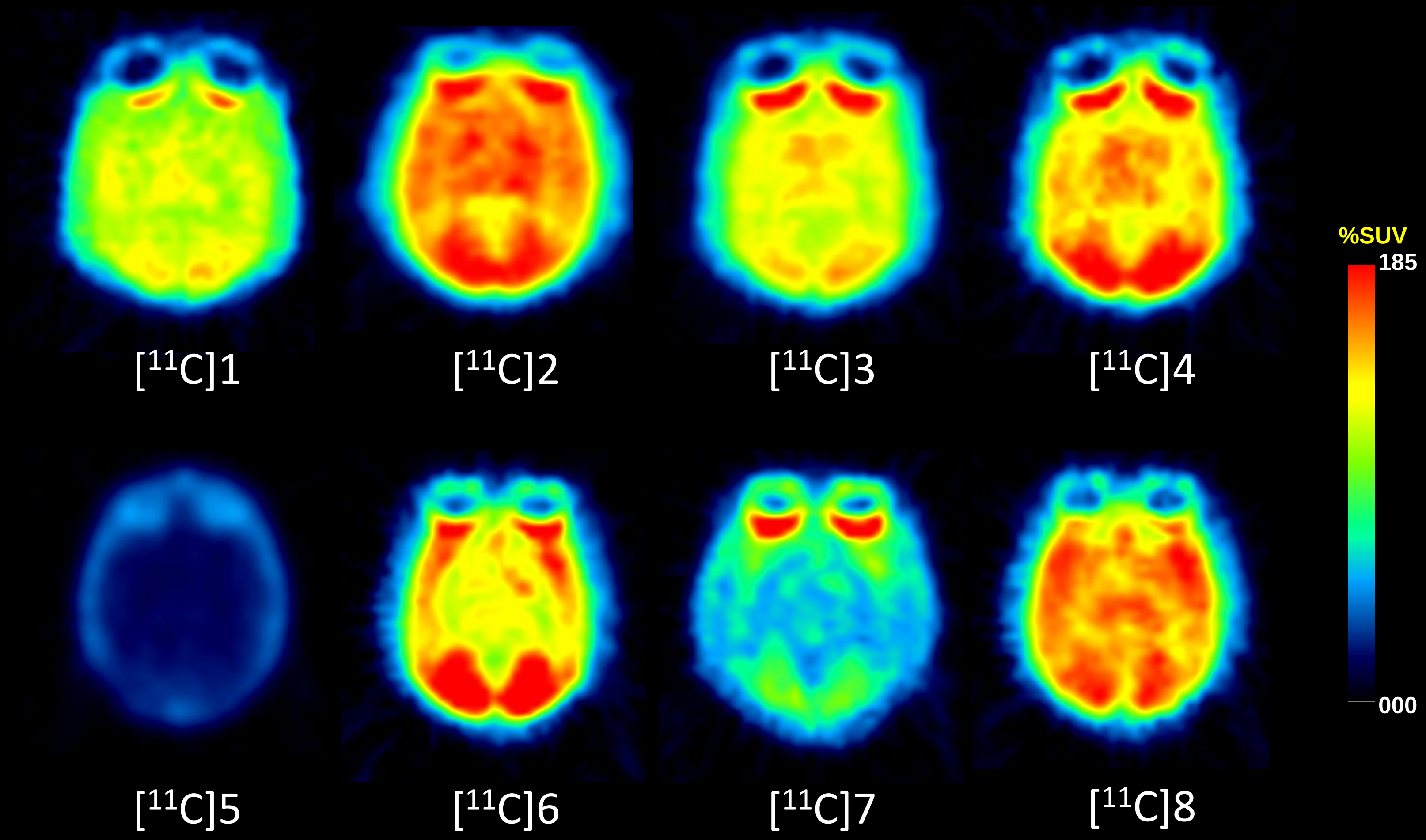


	R_1	R_2	*
[^{11}C]1.		F	Racemate
[^{11}C]2.		H	Racemate
[^{11}C]3.		H	R
[^{11}C]4.		H	S

	R_1	R_2	R_3
[^{11}C]5.		F	H
[^{11}C]6.		H	CH_3
[^{11}C]7.		H	H

[^{11}C]8.

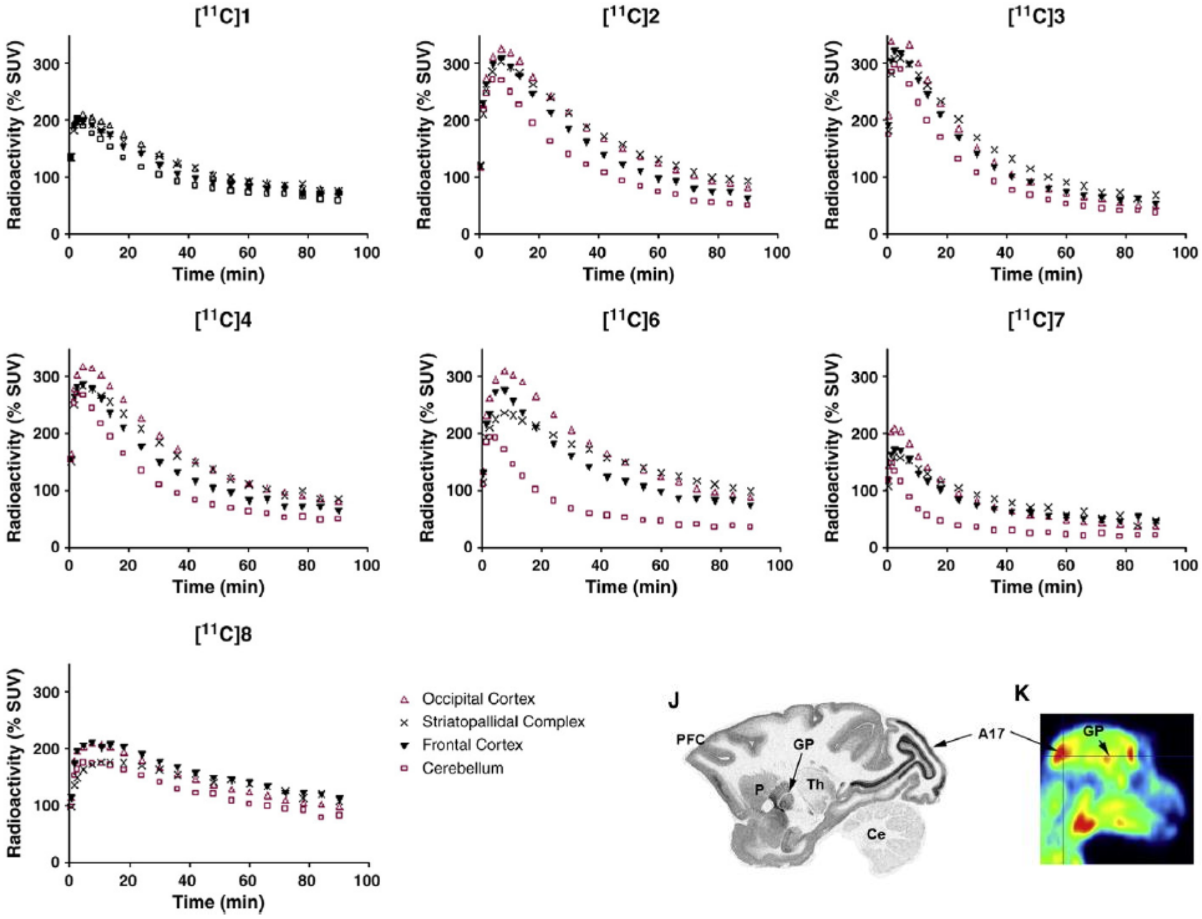
Development program 5-HT$_{1B}$ radioligand



Summation images: representing mean activity from 9-93 minutes

Development Program 5-HT_{1B} Receptor Radioligand

Comparison in NHP

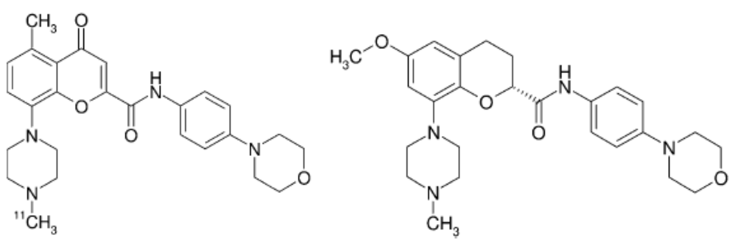


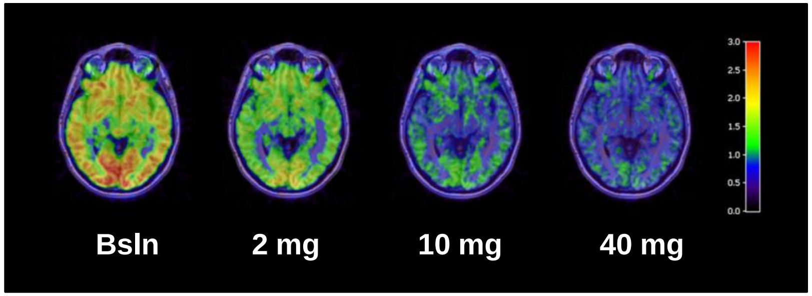
Andersson et al., 2011, *Nucl. Med. Biol.*

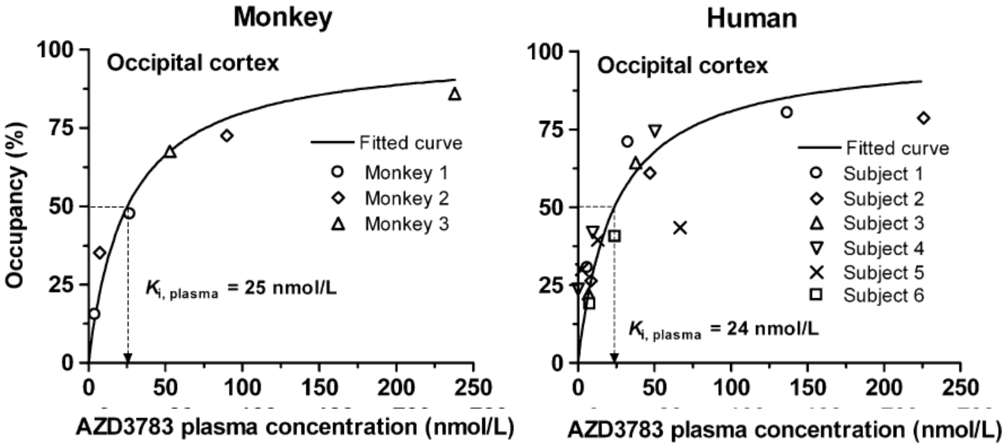
Validation of Drug Target Engagement

$5\text{-}HT_{1B}$ receptor occupancy by clinical candidate (AZD3783)

[11]C]AZ10419369 AZD3783







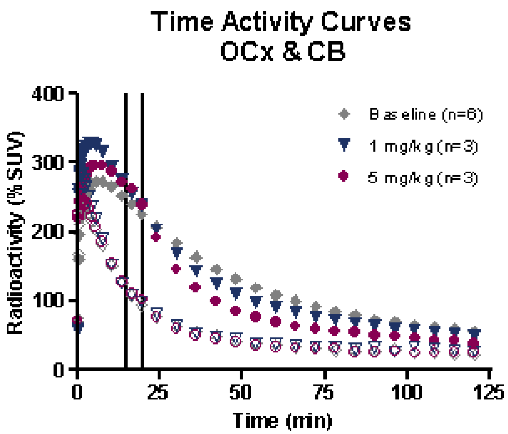
Predictive to human

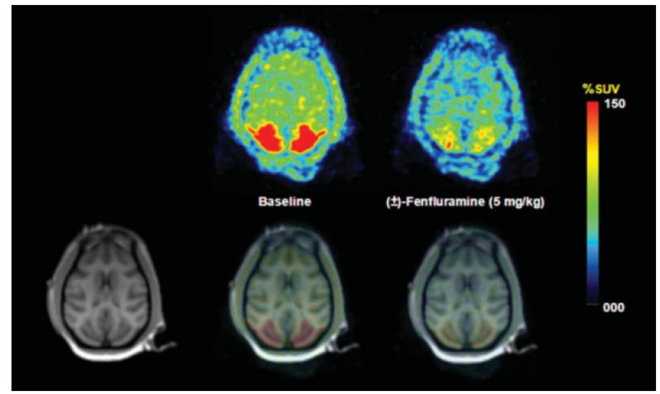
Varnäs et al., 2011, *Psychopharmacology*
Varnäs et al., 2013, *Cephalalgia*

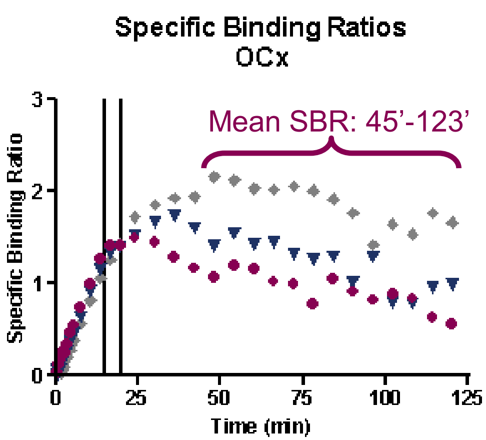
Fenfluramine-Induced Serotonin Release Decreases [^{11}C]AZ10419369 Binding to 5-HT$_{1B}$-Receptors in the Primate Brain

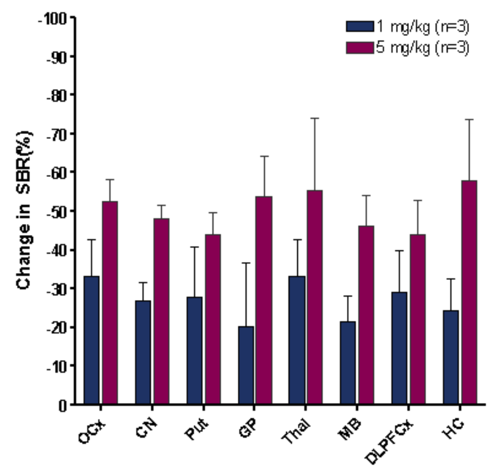
S.J. FINNEMA,[1*] A. VARRONE,[1] T.J. HWANG,[1] B. GULYÁS,[1] M.E. PIERSON,[2]
C. HALLDIN,[1] AND L. FARDE[1,3]

SYNAPSE 64:573–577 (2010)



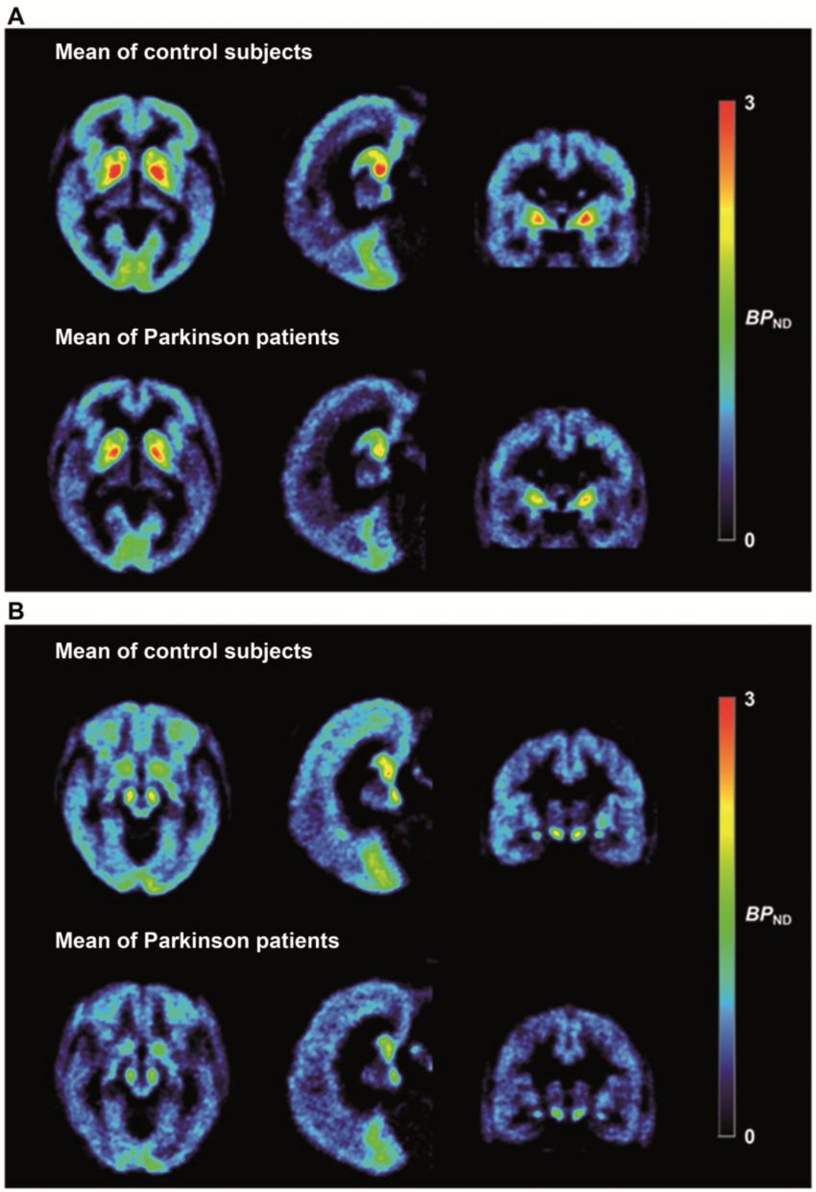


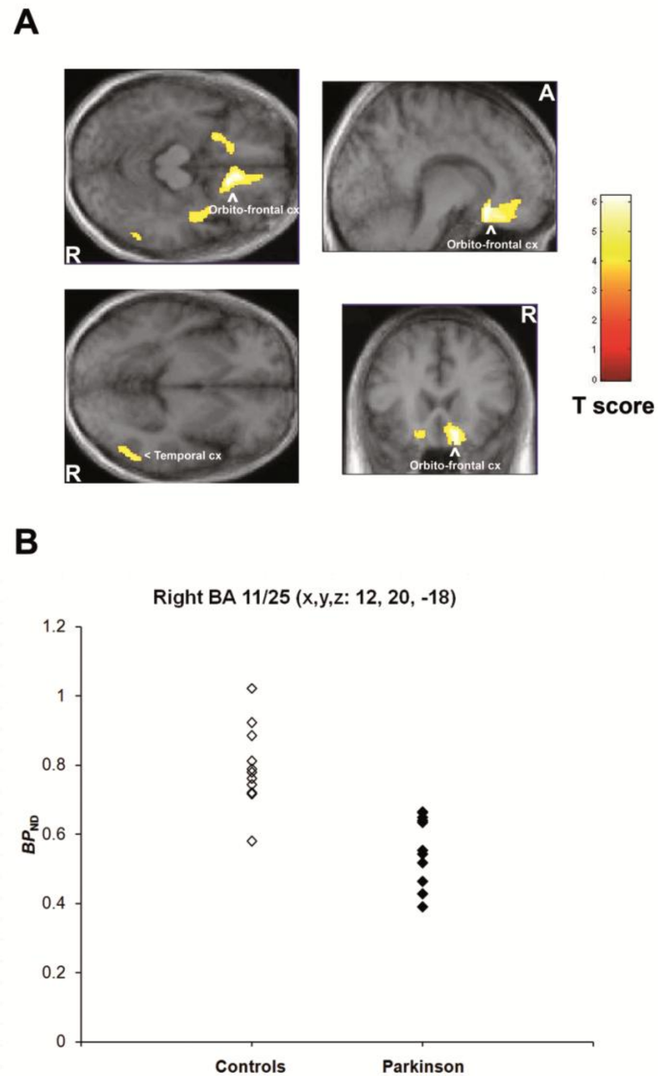




Measure serotonin release

5-HT_{1B} receptor imaging in PD – [^{11}C]AZ10419369

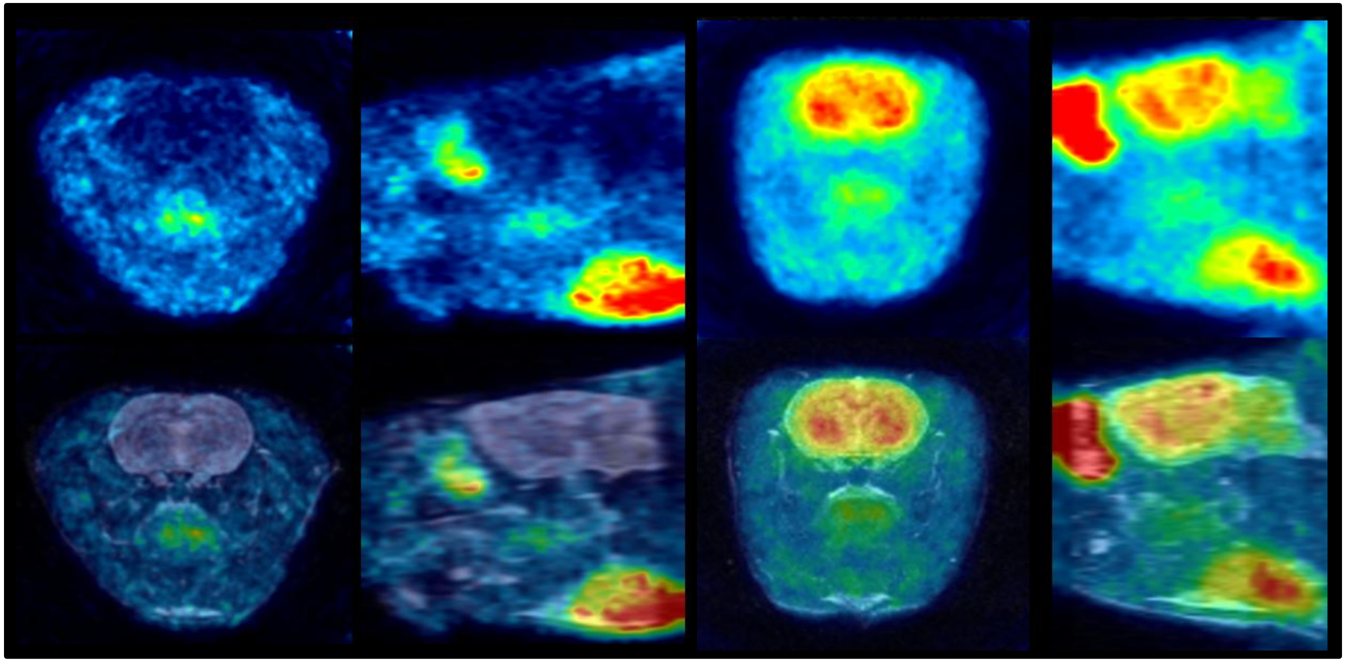




A PET radioligand for Parkinson's disease

Varrone et al. Neurobiology of aging 2014

Cyclosporin effects on PGP activity in rat: [^{11}C]AZ10419369



Baseline
[^{11}C]AZ10419369
(injected radioactivity: 24.7 MBq)

Pre-treatment with Cyclosporine
[^{11}C]AZ10419369
(injected radioactivity: 19.8 MBq)

Toth et al., *EJNM Research 2015*

<u>Why</u> is PET a ”hot” methodology in CNS drug discovery development?

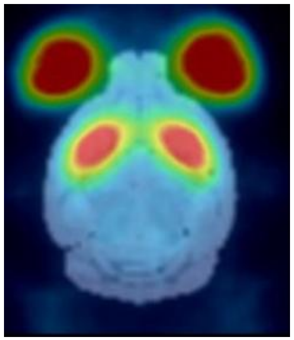
Several major applications

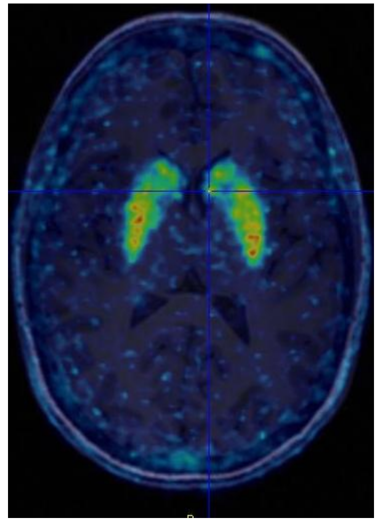
- Microdosing
- Drug occupancy at target of interest
- Imaging biomarkers of pathophysiology

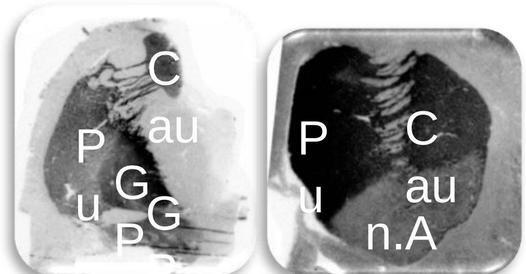
Success story 2
PD

En example of Translational Validation

microPET



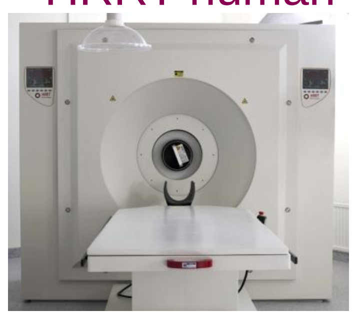


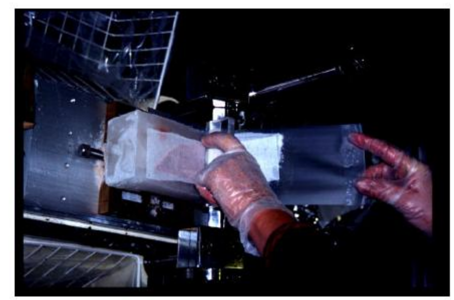


axial coronal

ARG

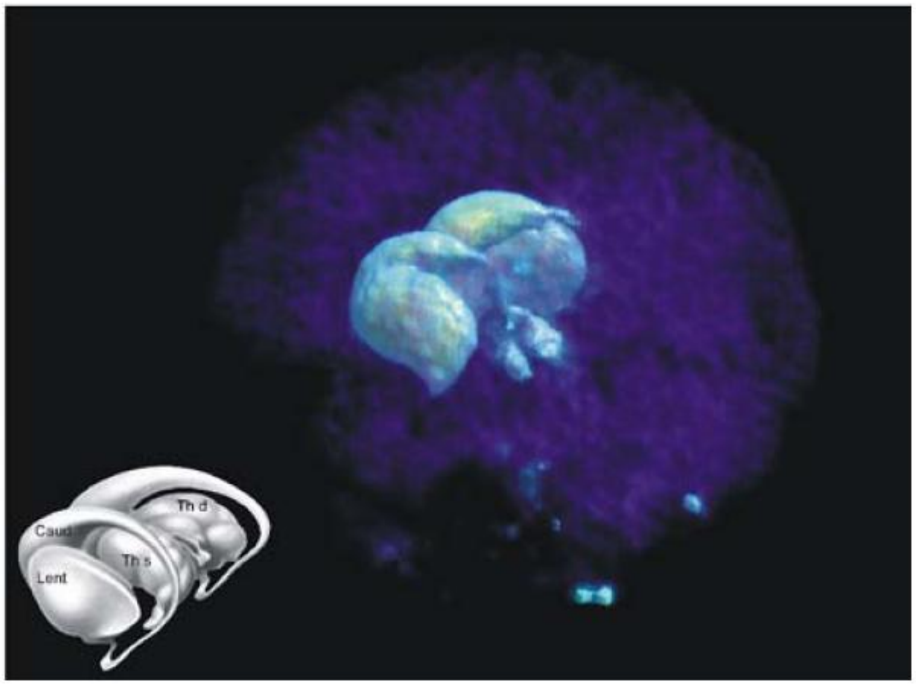
HRRT human



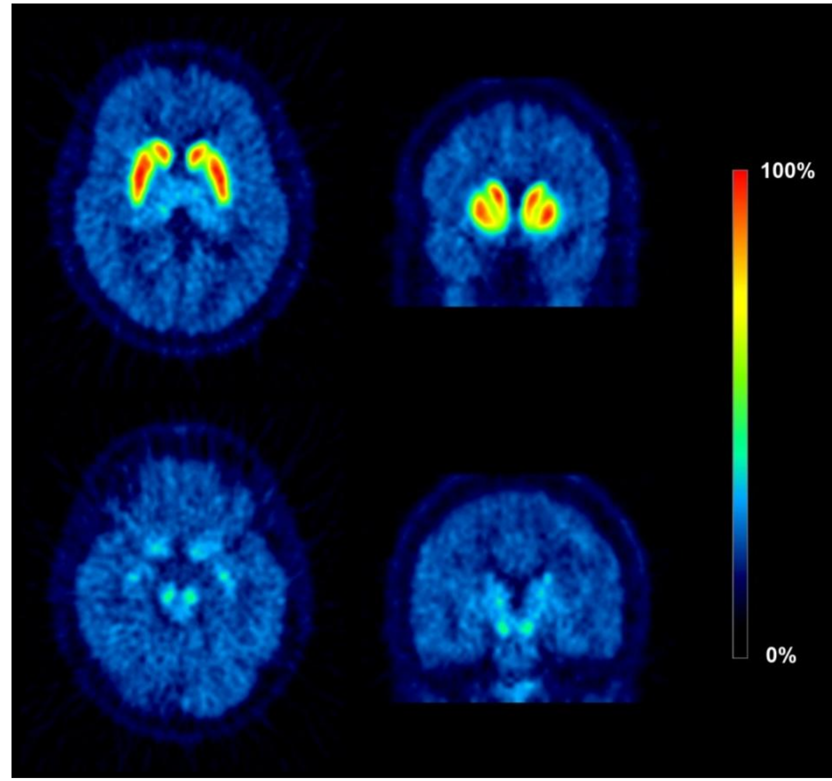


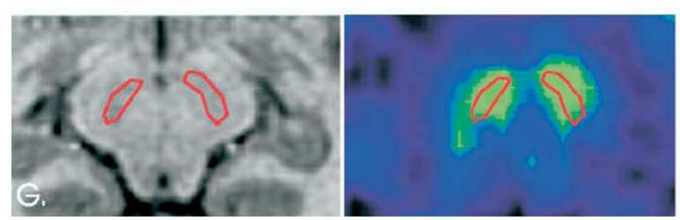
[^{18}F]FE-PE2I for DAT

DAT – [^{11}C]PE2I



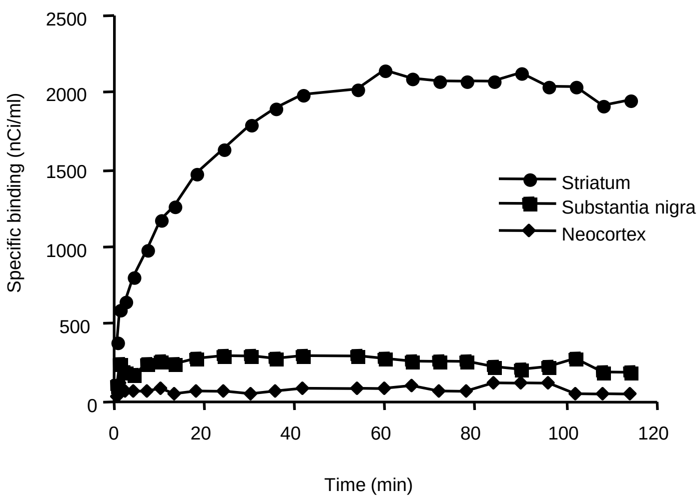
Halldin C, EJNMMI 2003;30:1220-1230



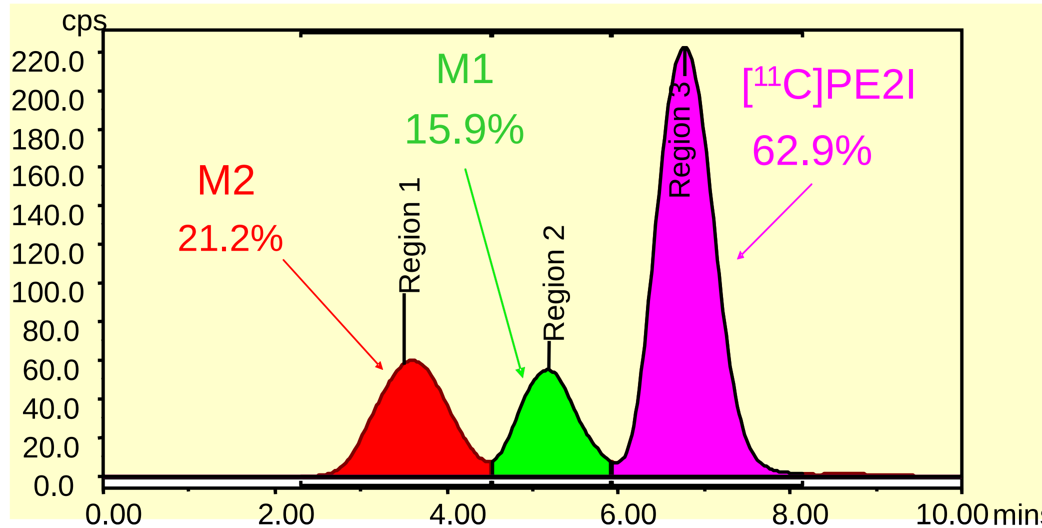


Jucaite A, Biol Psychiatry 2005;57:229–238
ADHD

[^{11}C]PE2I
Possible limitation: late equilibrium

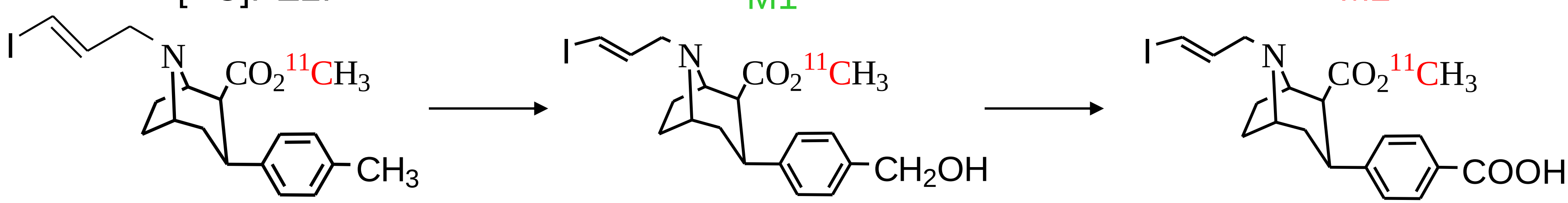


Metabolism of [^{11}C]PE2I



Metabolism analysed by LC-MS-MS

[^{11}C]PE2I M1 M2

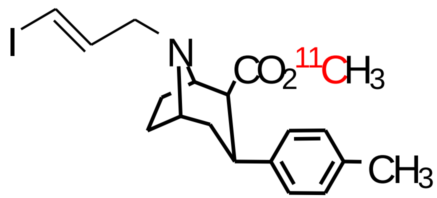


Shetty HU, et al., EJNMMI, 2007, NIMH, Karolinska Institutet

Non-human primates (rhesus monkey)

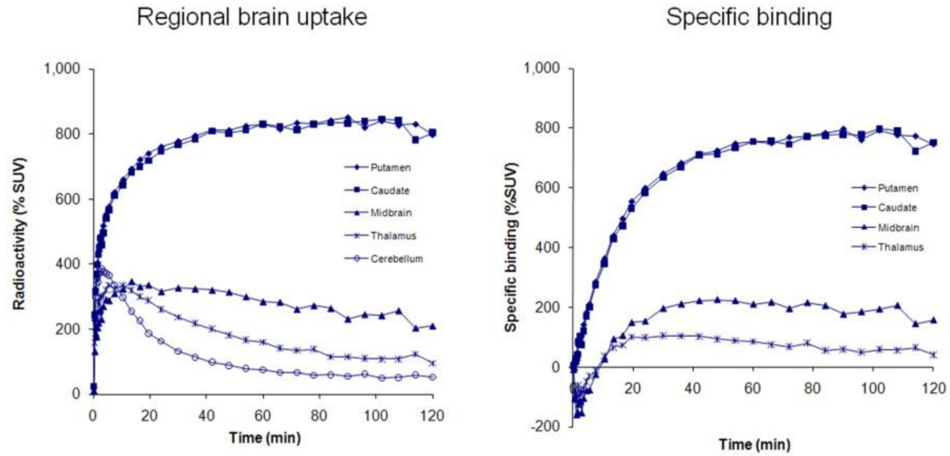
Karolinska Institutet

[^{11}C]PE2I

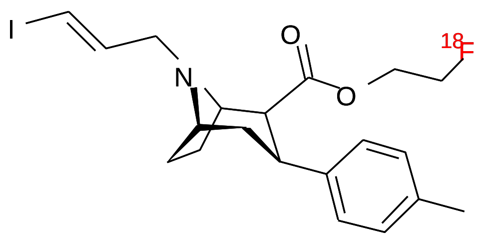


Well established DAT radioligand
- Slow kinetics
- Late peak equilibrium

^{11}C-PE2I

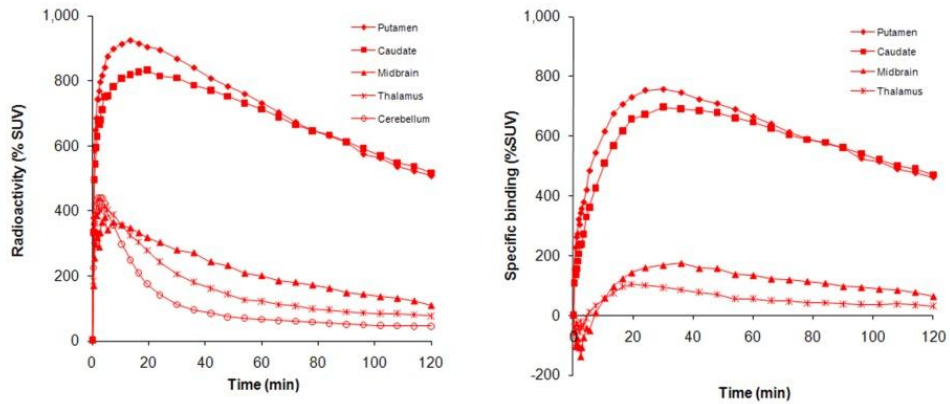


[^{18}F]FE-PE2I



- Faster kinetic properties
- Earlier peak equilibrium

^{18}F-FE-PE2I



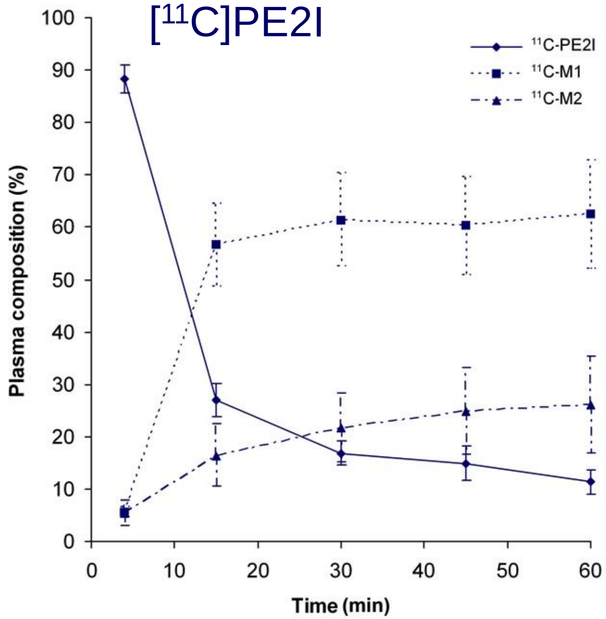
Varrone A et al. J Nucl Med 2011;52:132-9

- K_i of FE-PE2I at rodent DAT (tissue) was 12 ± 1.7 nM (n=3)
- K_i of FE-PE2I at rodent 5-HTT (homogenate) was > 1 mM (n=3)

Schou M et al. Bioorg Med Chem Lett. 2009;19:4843-5
Varrone A et al. Synapse 2009;63:871-80

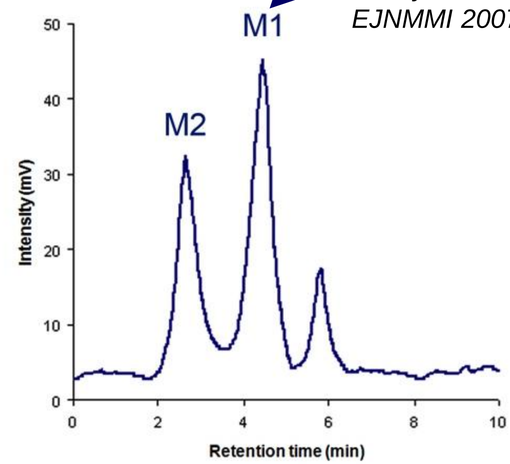
Metabolism of [11C]PE2I and [18F]FE-PE2I in rhesus monkey

A



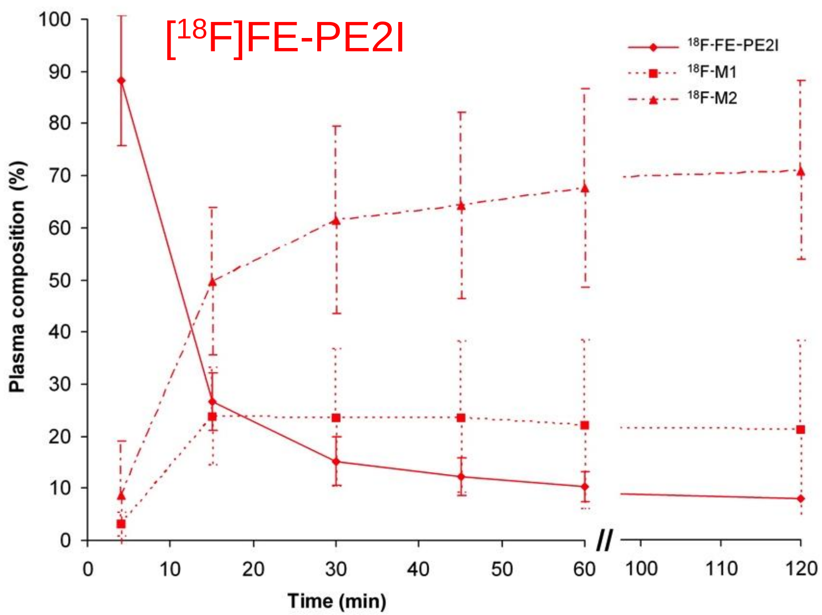
Metabolite entering the rat brain

Shetty HU et al.
EJNMMI 2007;34:667-678

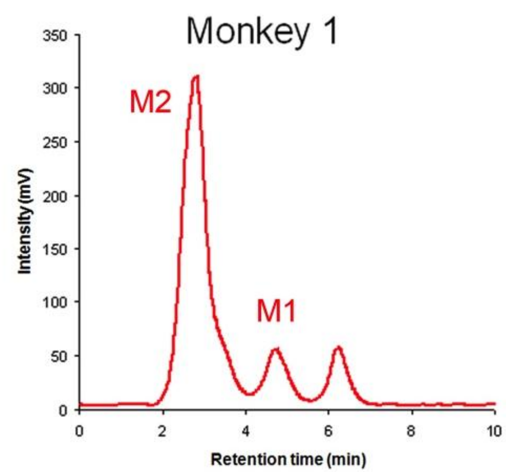


HPLC - 45 min

B



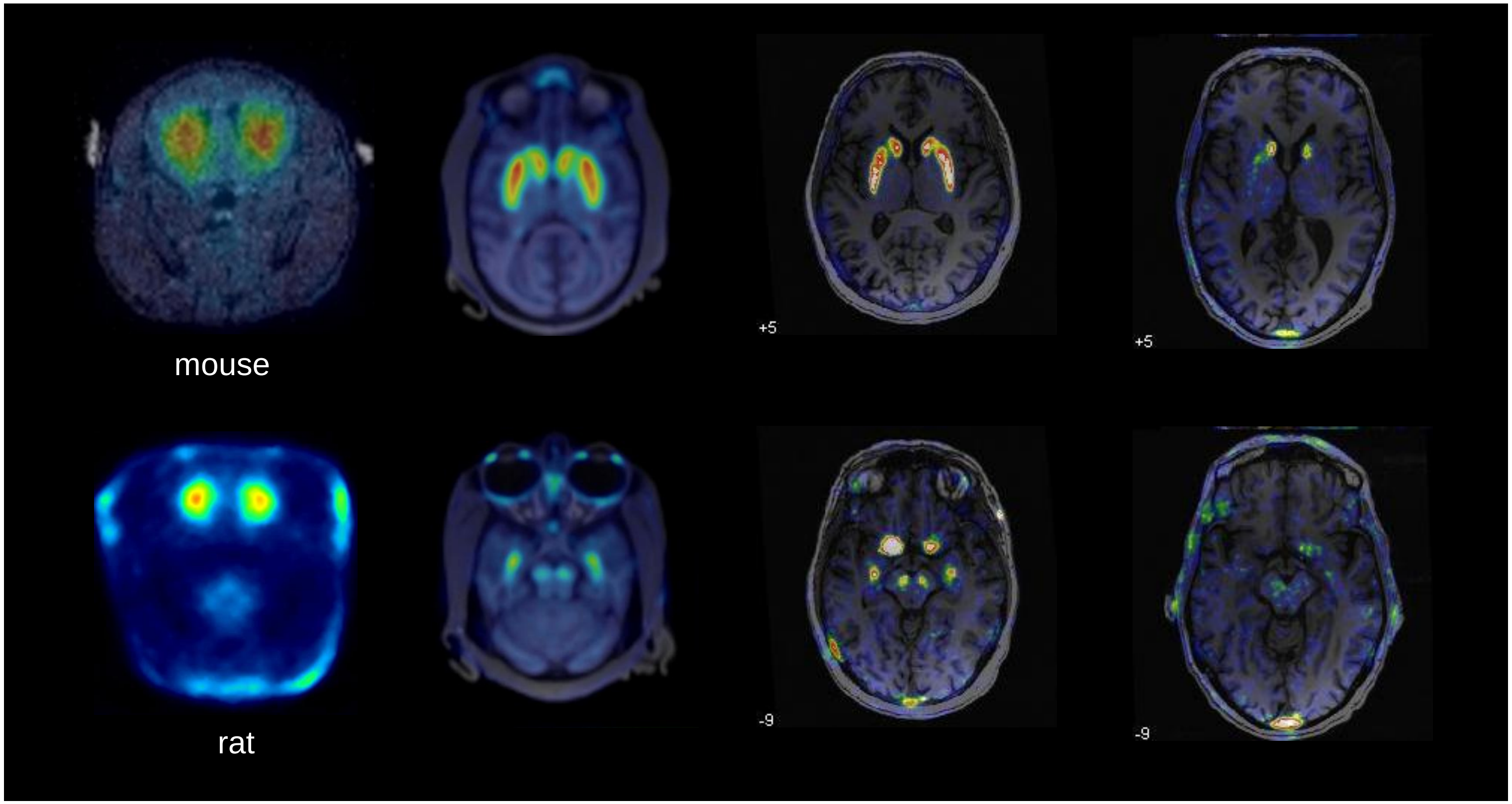
More favourable metabolism



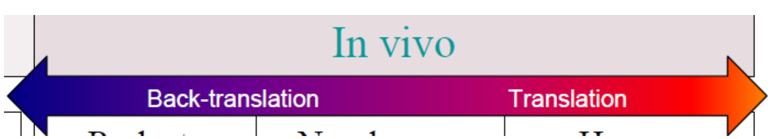
Varrone A et al. J Nucl Med 2011;52:132-9

En example of Applied Translational Coverage

[^{18}F]FE-PE2I



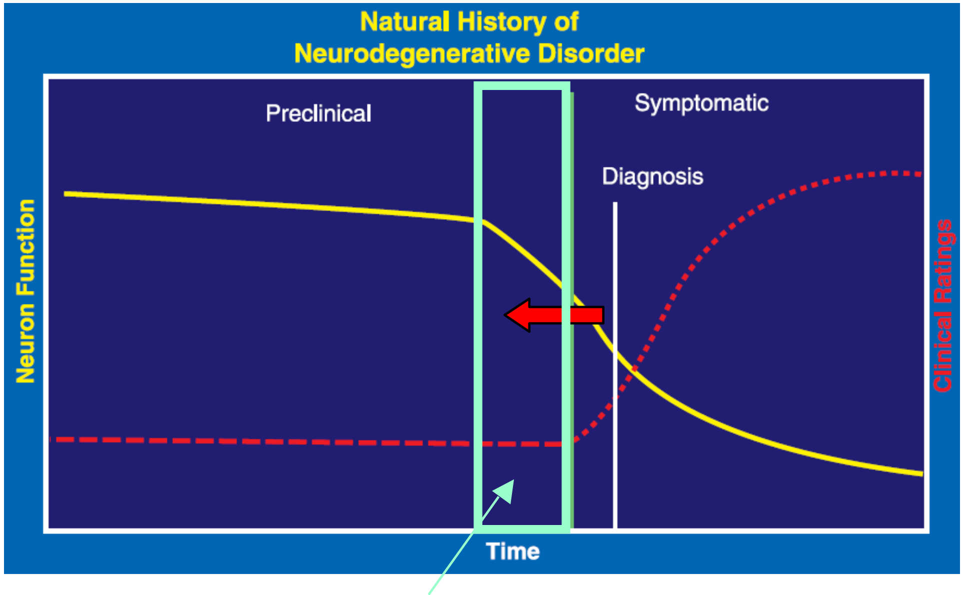
Rodents NHP Healthy control PD patient



Andrea Varrone

Success story 3
AD

Early diagnosis of neurodegenerative diseases



Asymptomatic phase

Decrease of neurotransmission parameters before
- the apparition of clinical signs

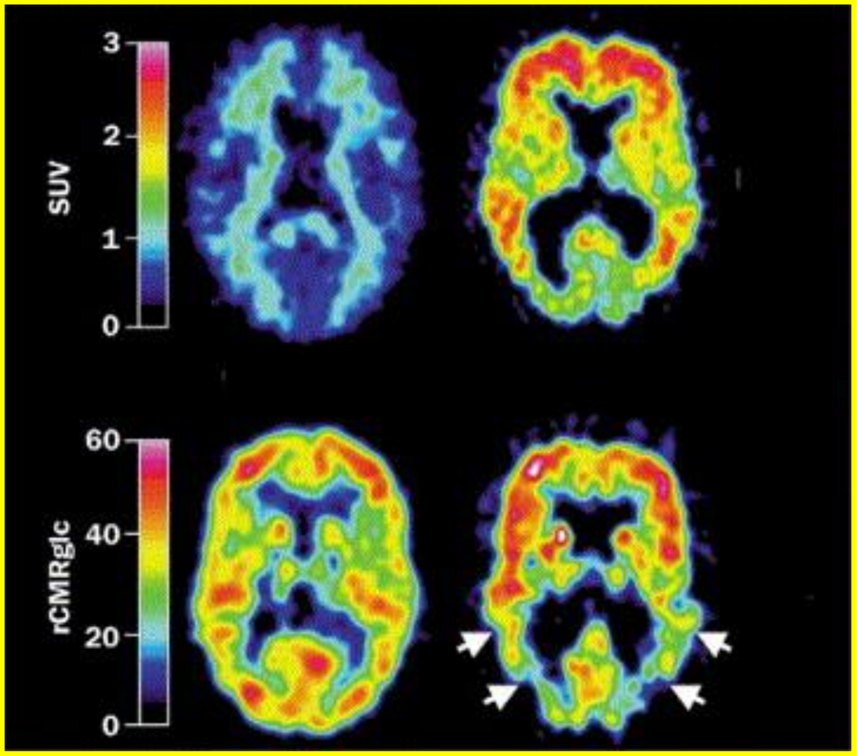
Asymptomatic phase

Possibility to perform diagnosis based on neurotransmission
during asymptomatic phase?

Modified from DeKosky and Marek, *Science 2003; 302:830*

Amyloid Imaging

First generation ligands



[11C]PIB

Klunk et al, Annals of Neurol. 55, 2004

Second generation ligands

Wishlist:

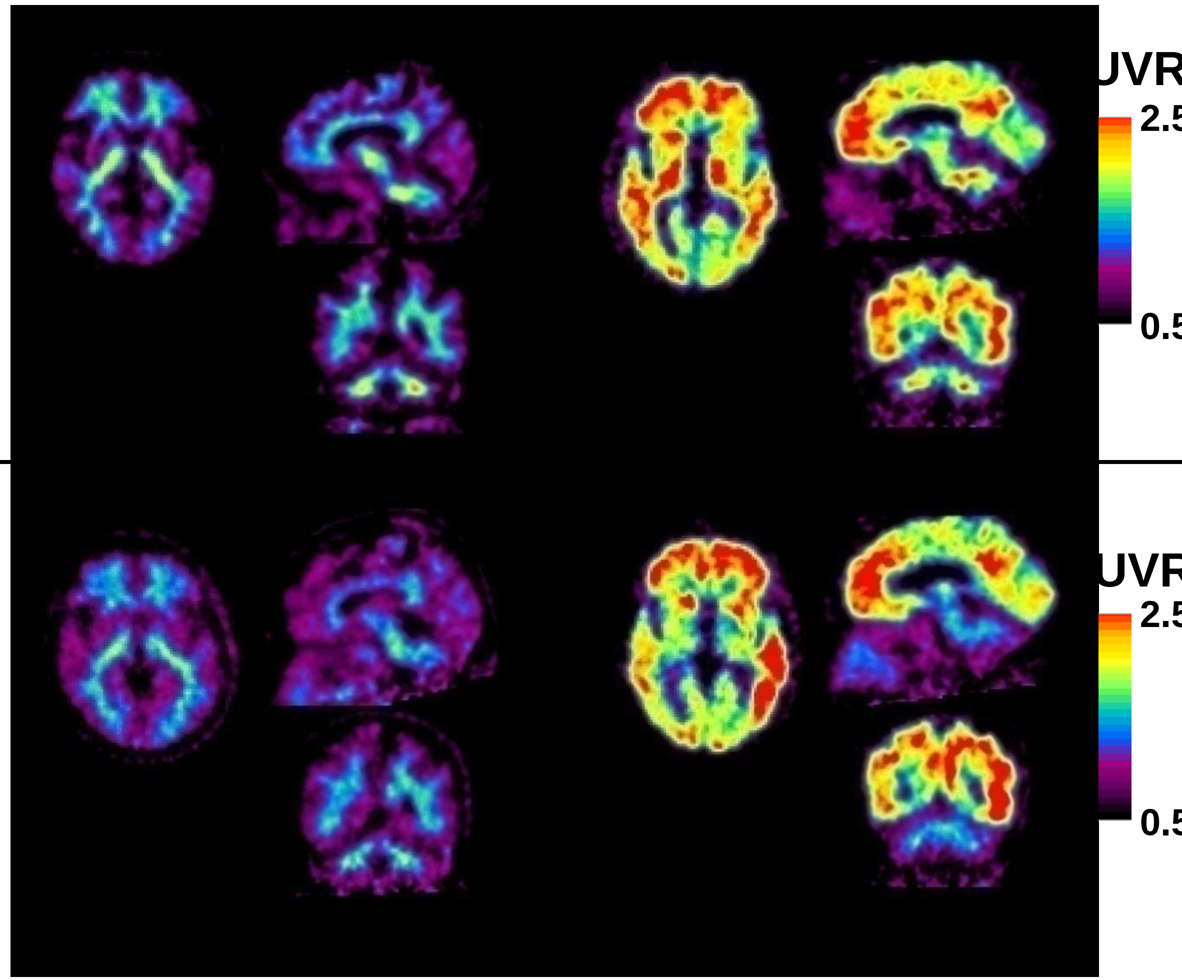
- Sensitivity for low amyloid plaque load
  - No white matter binding
  - Early detection of amyloid
  - Suitable for detailed mapping Regional analysis
- Reversible binding
  - Established approaches for valid quantification
- Excellent reliability
  - Evaluation of disease modifying therapies

Comparison of [^{18}F]3'-F-PiB and [^{11}C]PiB in the same control and AD subject

CONTROL AD

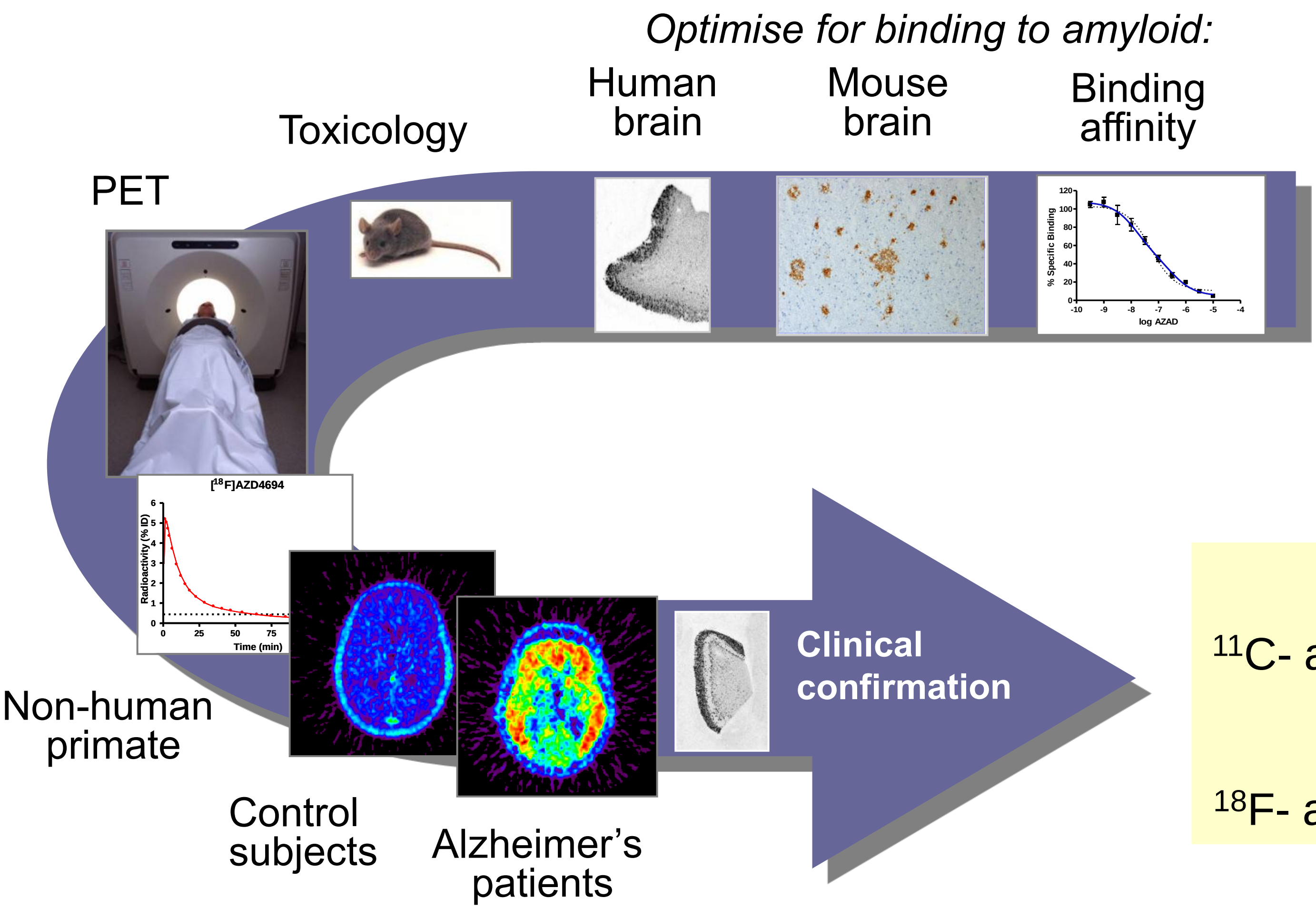
[^{18}F]3'-F-PiB or [^{18}F]GE-067

[^{11}C]PiB



Mathis et al, Pittsburgh, USA

Using rational design to design a second generation amyloid PET radioligands



Testing of 1,000's compounds since 2003

AZD2184

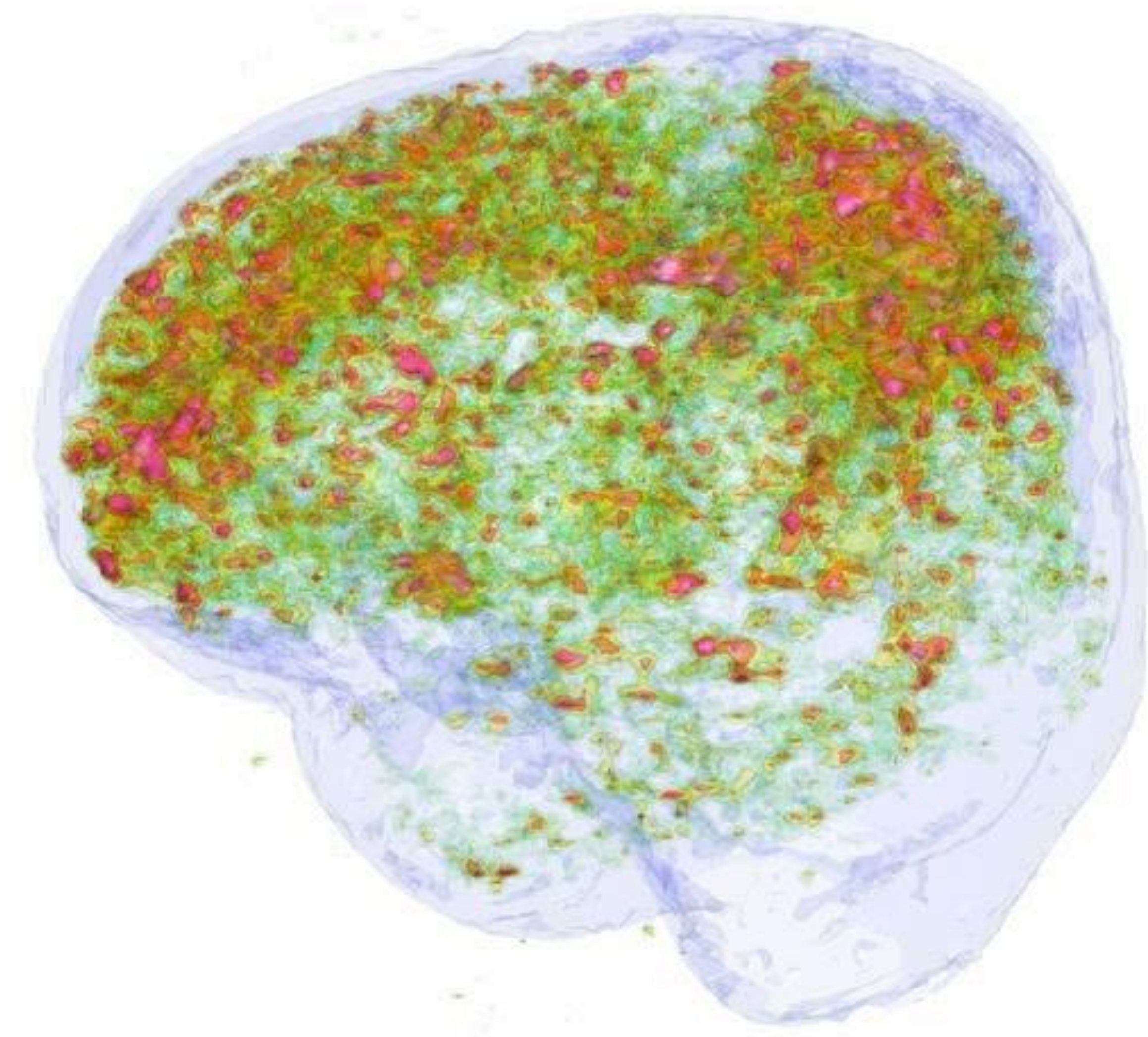
^{11}C- amyloid PET ligand

AZD4694

^{18}F- amyloid PET ligand

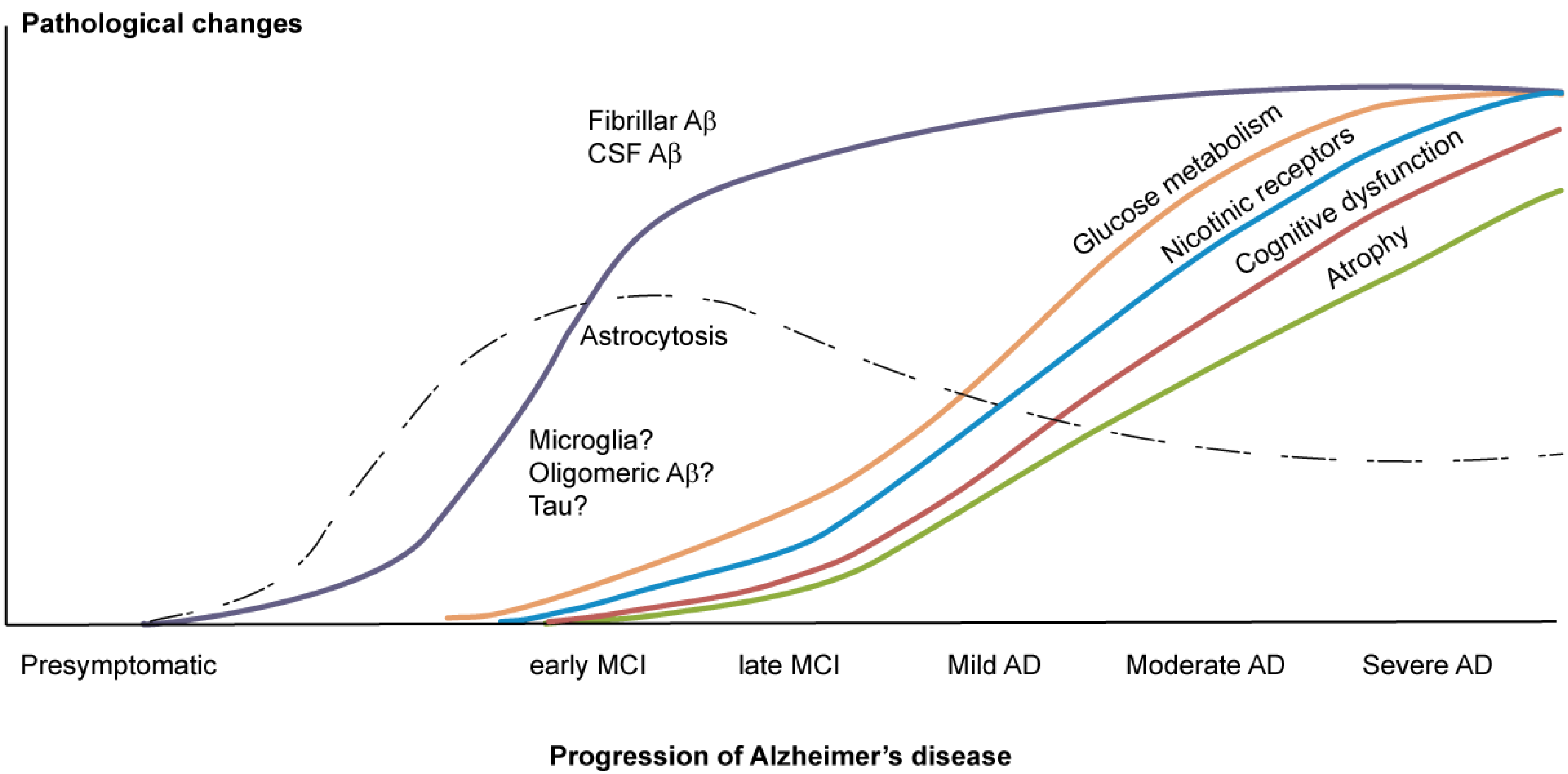
Marie Curie Award 2009

Quantification of [^{18}F]AZD4694 Binding: Parametric Imaging

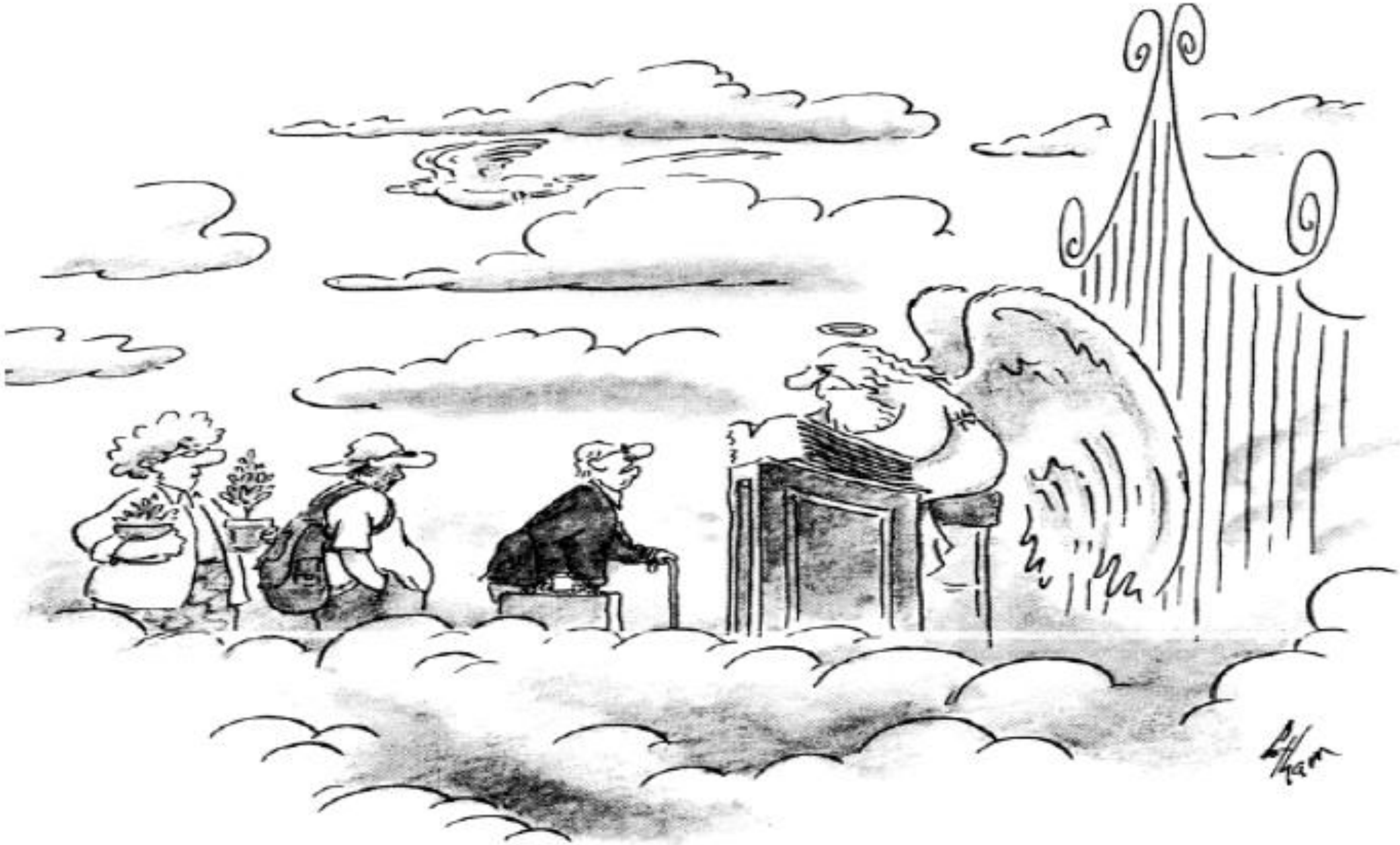


PET BP_{ND} parametric images in color overlaid on MR images in grey scale. Parametric images produced with SWAPI fitting RefLogan between 30-90 min post dose

Tentative time course pathological processes in AD



Nordberg Alzheimer's Research & Therapy 2011, **3**:34



"I'd have been here sooner if it hadn't been for early detection."

Acknowledgements

Karolinska Institutet PET Centre