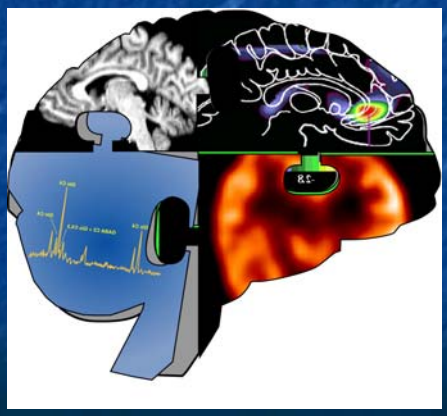
Discovery of PET Radioligands for Human Brain Imaging *In Vivo*



Victor W. Pike PhD
Molecular Imaging Branch (MIB)
NIMH, Bethesda, MD, USA

Why do we need radioligands for *in vivo* brain imaging?

- For investigation of involvement of brain proteins (*e.g.* transporters, receptors, enzymes, plaques) in neuropsychiatric disorders in living systems – especially human subjects.
- For expediting drug discovery and development in the treatment of neuropsychiatric disorders *e.g.* determining dose and dosing interval; providing biomarkers for drug efficacy.

What radionuclide imaging modalities are available for molecular imaging *in vivo*?

- Single Photon Emission Computed Tomography (SPECT)

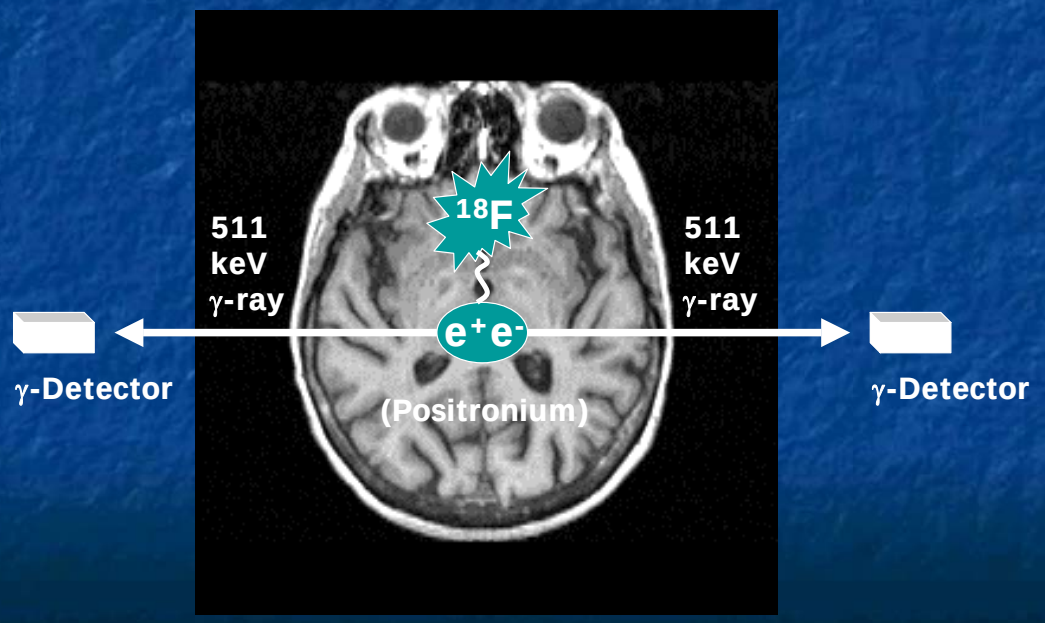
  – principally uses γ-emitting radiotracers labeled with ^{99m}Tc or ^{123}I
- Positron Emission Tomography (PET)

  - principally uses radiotracers labeled with positron-emitting ^{11}C or ^{18}F

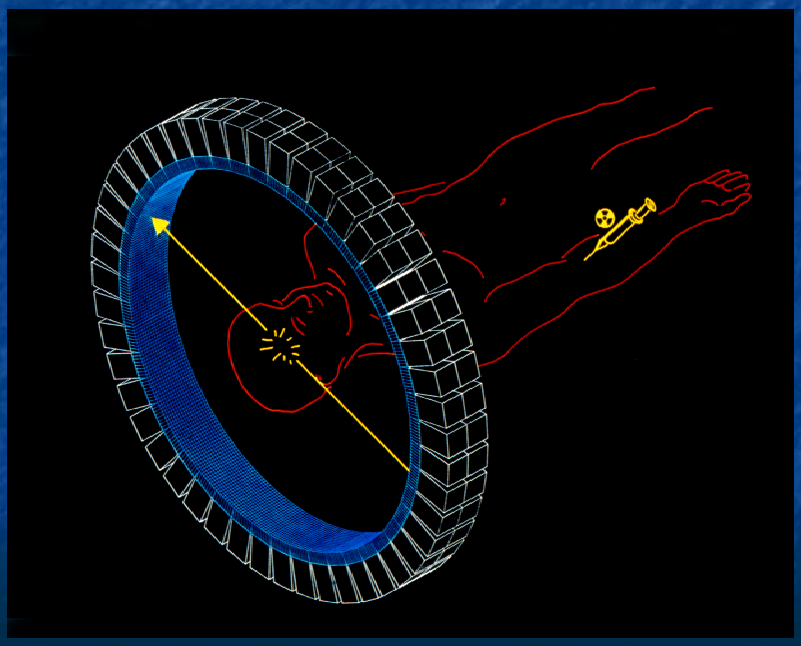
Comparison of PET with SPECT

	PET	SPECT
Resolution (spatial)	2 mm	7 mm
Resolution (time)	sec/min	min
Sensitivity (concn.)	pM	pM
Cost	High	Lower
Quantification	Good	Less Good
Availability of isotopes	Cyclotron	Commercial

Physical basis of positron emission tomography (PET)



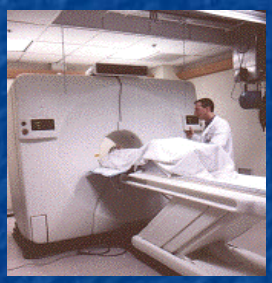
Typical PET camera configuration



State-of-the-art PET

- Primate (human, monkey)

e.g. NIH GE Advance camera
Spatial resolution: 5 – 8 mm
Time resolution: seconds
Sensitivity: 0.12% (2D); 0.7% (3D)
Axial field of view: 15 cm



- Small animal (rat, mouse)

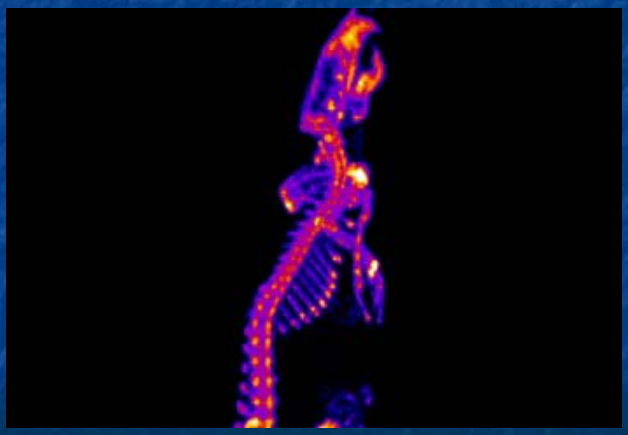
e.g. NIH ATLAS camera
Spatial resolution: 1.2 – 1.8 mm
Time resolution: seconds
Sensitivity: 2.7%
Axial field of view: 2.0 cm

ATLAS Camera - [^{18}F]fluoride ion uptake in rat skeleton

ATLAS camera developed at NIH by:

Mike Green &

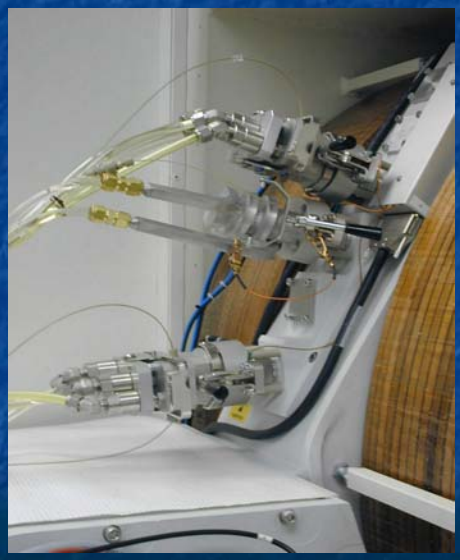
Jurgen Seidel



Chemical basis of PET – positron-emitting tracers

- Oxygen-15
  $t_{1/2}$ = 2.03 min; extremely limited chemical possibilities
- Nitrogen-13
  $t_{1/2}$ = 9.97 min; very limited chemical possibilities
- Carbon-11
  $t_{1/2}$ = 20.4 min; versatile chemical possibilities
- Fluorine-18
  $t_{1/2}$ = 109.8 min; quite versatile chemical possibilities

Cyclotron production of positron-emitters



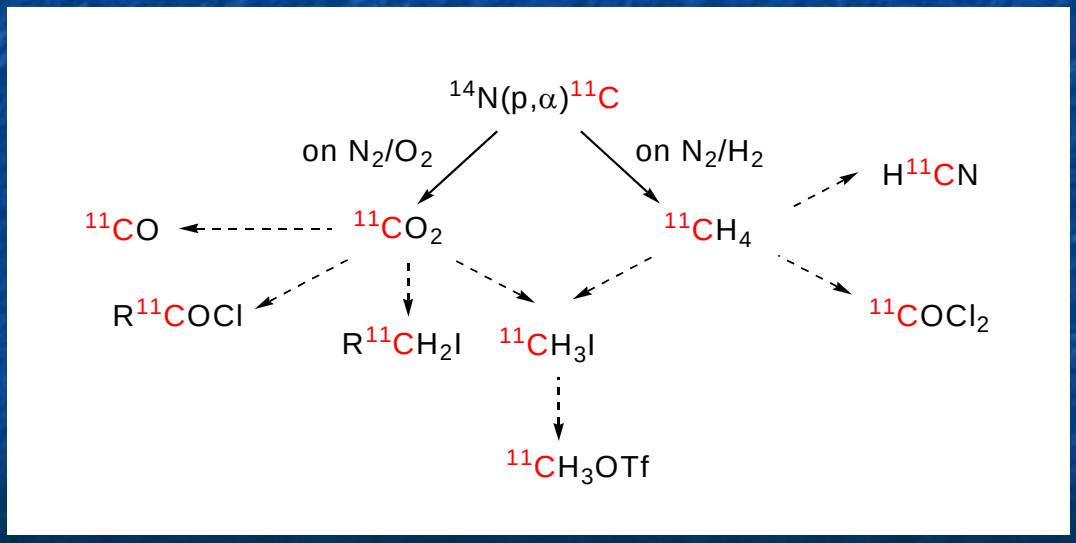
Selection criteria for target protein to be imaged

- Implicated in one or more neuropsychiatric conditions (*e.g.* drug target; implications from *post mortem* studies of human brain…) *i.e.* of interest to *basic science* and/or *drug discovery* or *drug development*.
- Concentrated in brain with a discrete normal distribution (ideally, also including a region of absence).
- Containing a well-characterized (preferably unique) small molecule binding site.

[^{11}C]Radioligands

- In principle, carbon-11 may be introduced into any organic compound, whether endogenous or exogenous.
- Suitable for monitoring 'fast' kinetics.
- High practical specific radioactivity is attainable (5–50 Ci/μmol).
- Versatile radiochemistry now exists for [^{11}C]radioligand production.

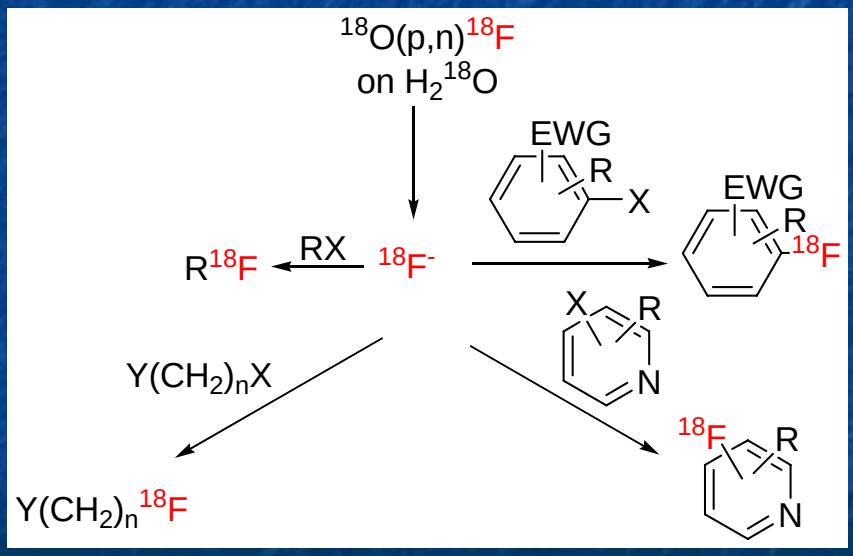
Carbon-11 Radiochemistry



[^{18}F]Radioligands

- Fluorine-18 may 'mimic' H or OH in organic compounds.
- Suitable for monitoring 'slower' kinetics.
- High practical specific radioactivity possible (10–20 Ci/μmol).
- Fluorine-18 may be produced in very high activity amounts (>10 Ci per batch).
- 110 min half-life permits [^{18}F]radioligand distribution to remote users.
- Quite versatile radiochemistry now exists for preparing [^{18}F]radioligands.

Fluorine-18 radiochemistry

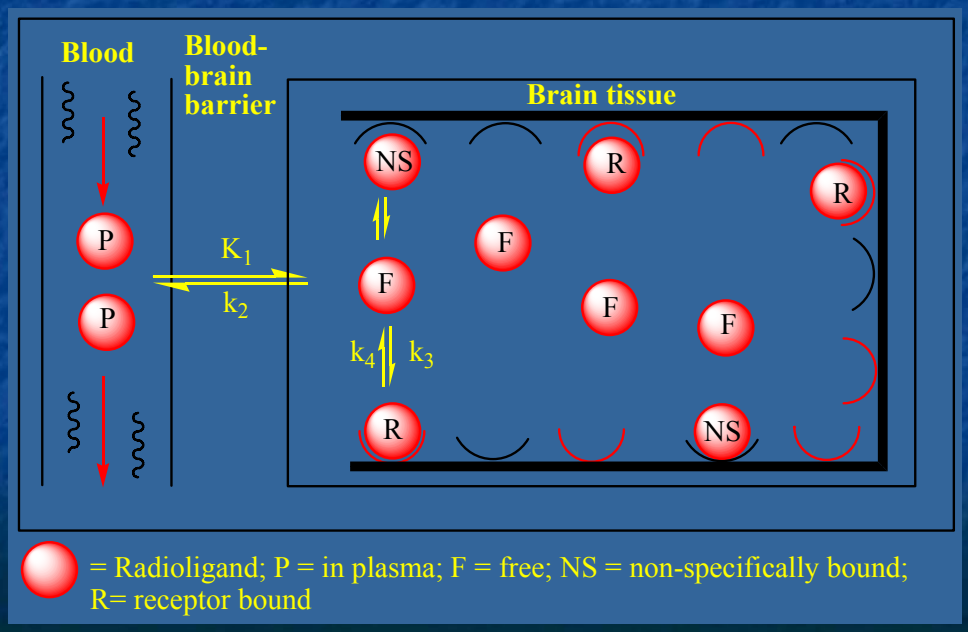


EWG = Electron withdrawing group; X = leaving group; Y = halo or tosyl

Biological basis of PET: mainly protein-ligand interactions

- Enzyme-substrate
- Enzyme-inhibitor
- Transporter-inhibitor
- Transporter-substrate
- Receptor-ligand (agonist/antagonist)
- Binding site-ligand

What will the PET camera see every few seconds?



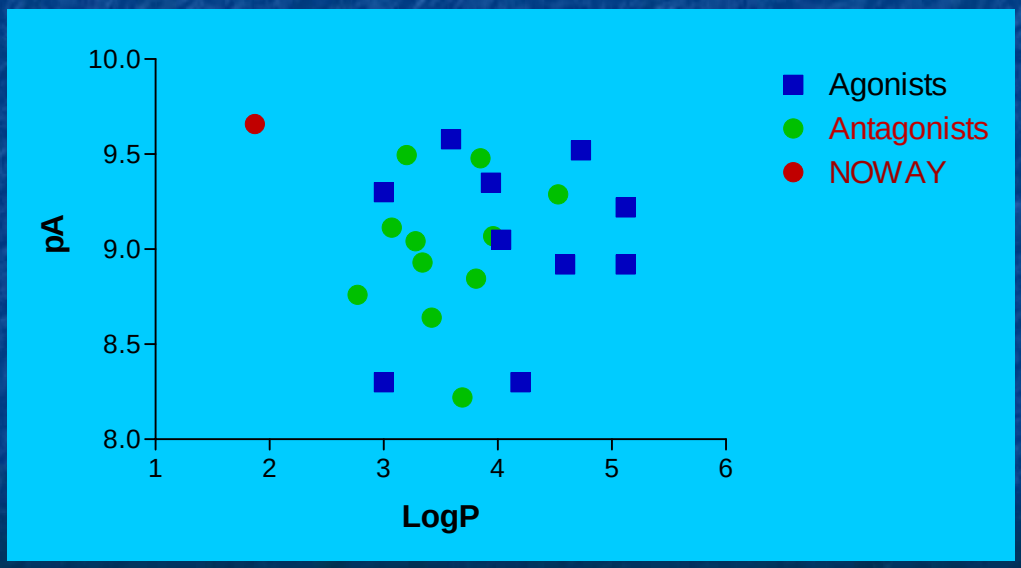
General requirements for candidate PET radioligands

- Known intrinsic activity (agonist or antagonist for GPCR)
- High binding potential for target receptor (B_{max}/K_d >> 1) *i.e.* usually very high affiinity
- Selectivity for target protein (receptor, transporter..)
- Ability to penetrate blood-brain-barrier (BBB); lack of substrate behavior for efflux pumps such as P-gp
- Moderate lipophilicity for low protein binding and low non-specific binding in brain
- No labeled metabolites able to penetrate BBB
- Kinetics allowing quantification of target
- Amenability to labeling with ^{11}C or ^{18}F
- Lack of pharmacological effect at low dose.

Intrinsic activity: antagonist *versus* agonist for GPCR targets?

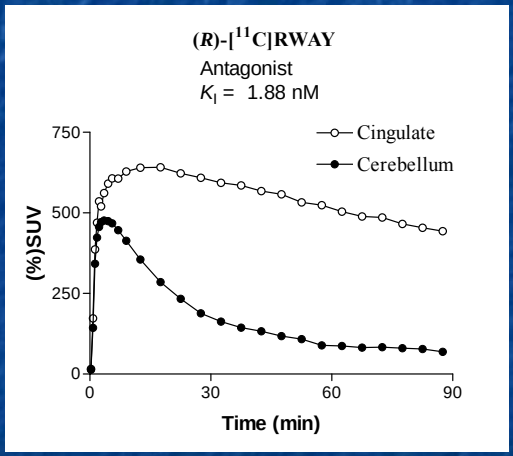
- For GPCR targets, antagonist will bind all target receptors
- Agonist is expected to bind only G-protein coupled receptors (a sub-population)

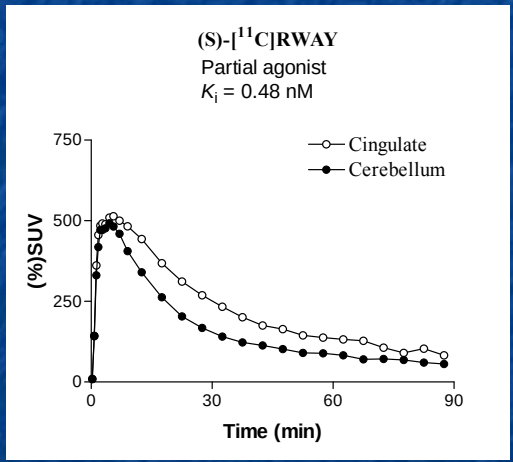
5-HT_{1A} Receptor radioligands – antagonists *versus* agonists



From data mainly compiled in review by Pike V.W. *et al., Progr. Med. Chem.*, (2001) 38, 189-247

5-HT_{1A} Receptor signal from [^{11}C]RWAY in monkey depends on enantiomer and its intrinsic activity



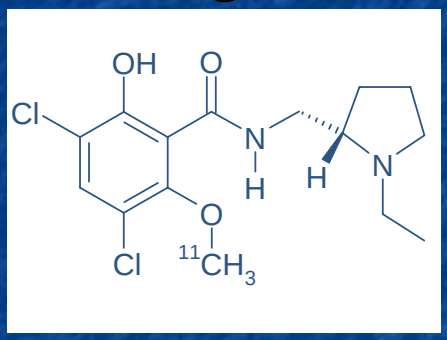


Magnitude of signal from, 5-HT_{1A} receptors in cingulate appears to depend on intrinsic activity, not affinity or other parameters (metabolism, lipophilicity)

McCarron J.A. *et al.*, *Eur J. Nucl. Med. & Mol. Imaging*, In press.

Dopamine D_2 receptor PET radioligands

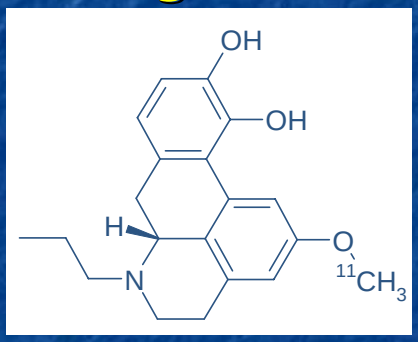
Antagonist



[^{11}C]Raclopride

Binds all D_2 receptors

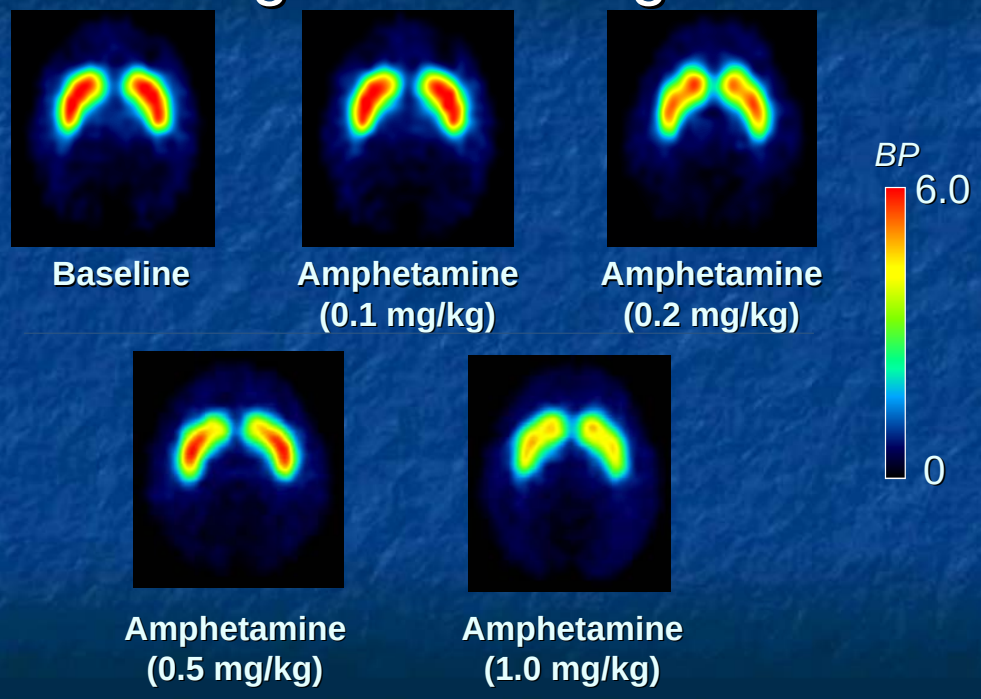
Agonist



[^{11}C]MNPA

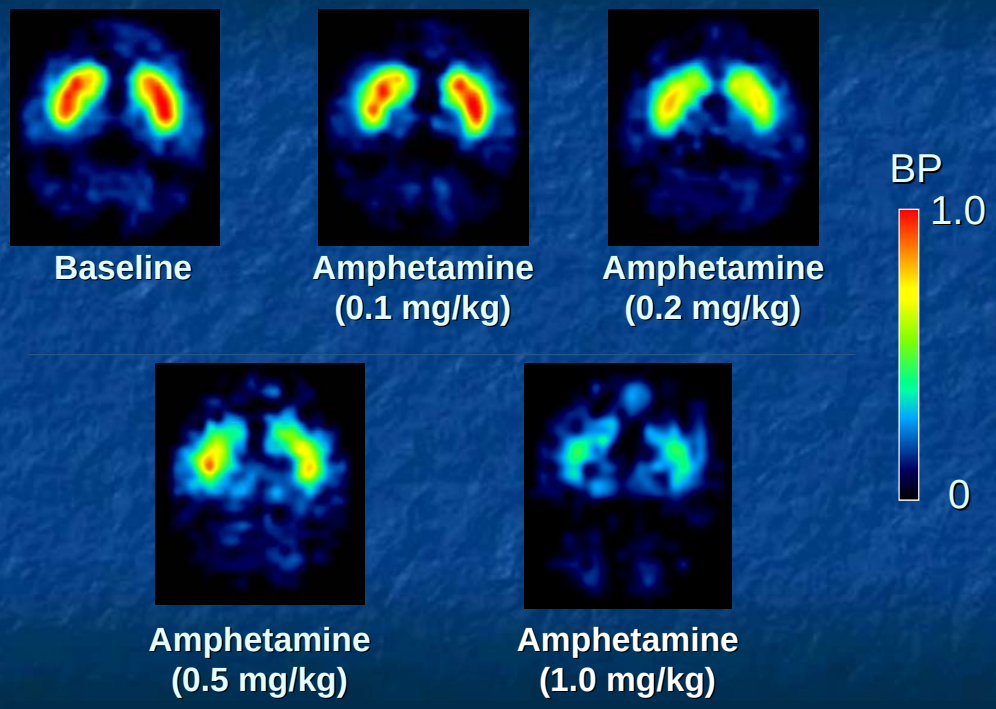
Binds G-protein coupled D_2 receptors

[^{11}C]Raclopride: D_2 receptor antagonist radioligand



Seneca N. *et al., Synapse* (2006) 59, 260-269

[^{11}C]MNPA: More Sensitive to DA



Seneca N. *et al., Synapse* (2006) 59, 260-269

Binding affinity: What is required?

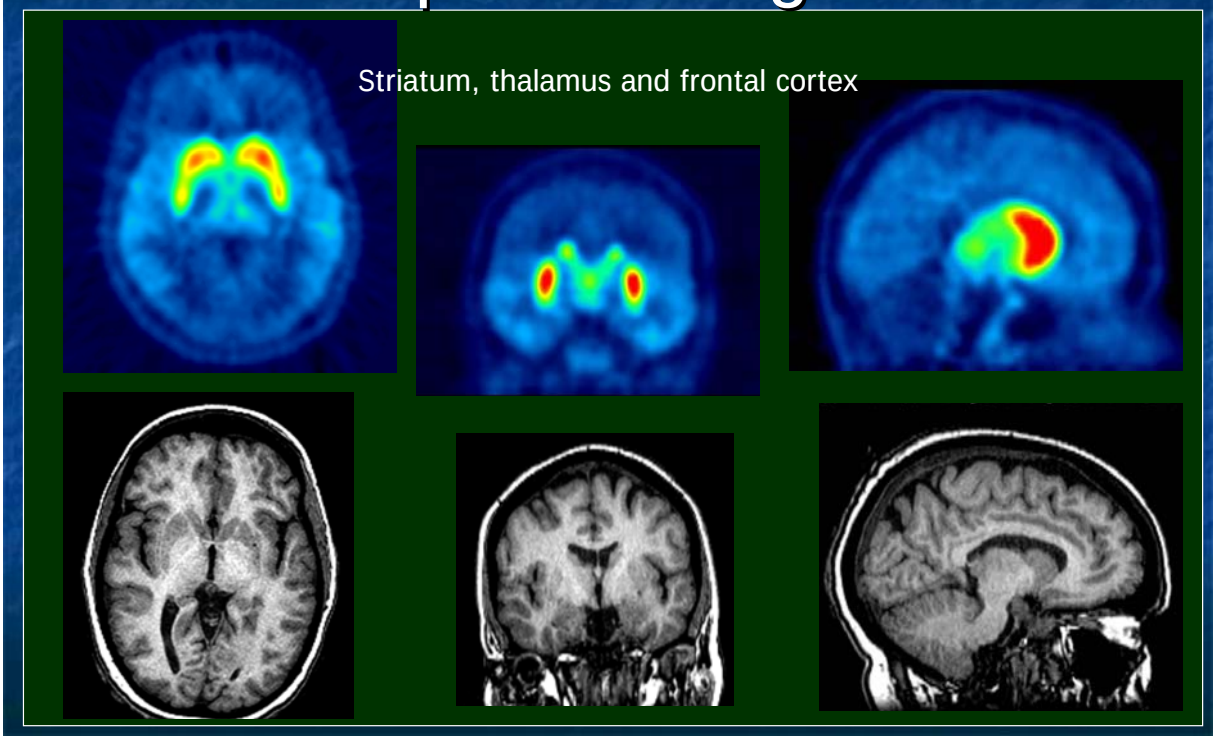
- Theoretically, B_{max}/K_D >>1 (each in units of nM)
- Example, D_2 receptor radioligands:

  [^{11}C]Raclopride (K_D = 1.3 nM)

  versus

  [^{18}F]Fallypride (K_D = 30 pM)

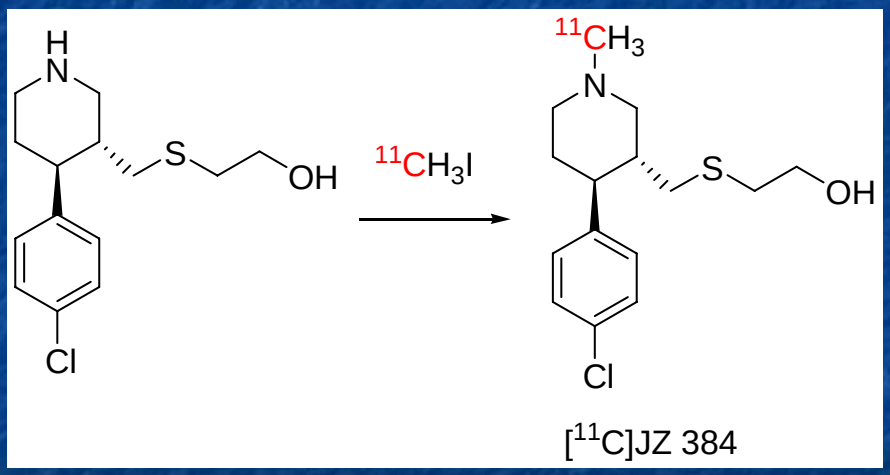
[^{18}F]Fallypride: a sensitive D_2 receptor radioligand



Selectivity: what is required?

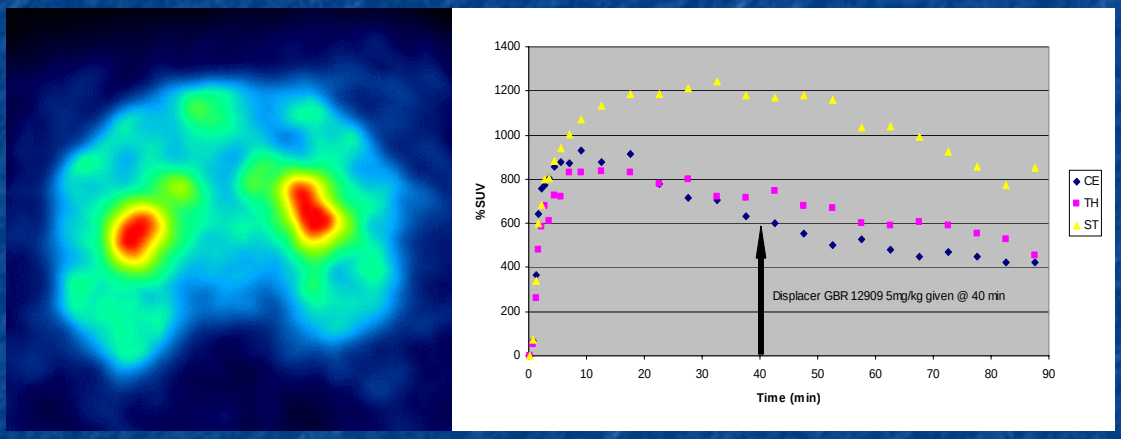
- Need to consider densities and location of 'off-target' sites for which the radioligand has affinity and also the affinity of the radioligand for these sites
- Example: prospective NET radioligands
  [^{11}C]JZ 384
  [^{18}F]MeNER-D_2

[^{11}C]JZ 384 – Prospective noradrenalin transporter (NET) radioligand?



K$_i$s (nM): DAT = 16; SERT = 158; NET = 0.9
i.e. DAT/NET = 17; SERT/NET = 176.

[^{11}C]JZ 384 in rhesus monkey brain



Transaxial PET image 30 min after injection of [^{11}C]JZ 384 and time-activity curves in Rhesus monkey brain

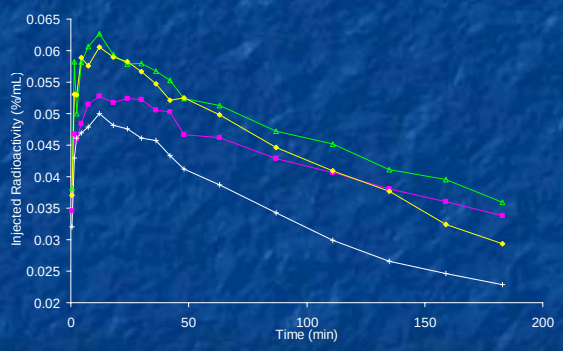
Musachio J.L. *et al.*, *Bioorg. Med. Chem. Lett.* (2006) 16, 3101-3104.

(*S,S*)-[^{11}C]MeNER and (*S,S*)-[^{18}F]FMeNER-D$_2$

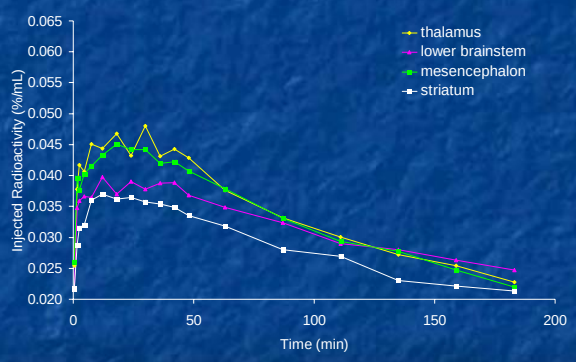
	(*S,S*)-[^{11}C]MeNER	(*S,S*)-[^{18}F]FMeNER-D$_2$
Ki at NET (nM)	2.50	3.1
Ki at SERT (nM)	310	> 1,000
Ki at DAT (n M)	> 1,000	> 1,000
cLogP	2.9	2.9

(*S,S*)-[^{18}F]FMeNER-D$_2$ - baseline *versus* DMI challenge

Baseline



DMI 5 mg/kg *i.v.*

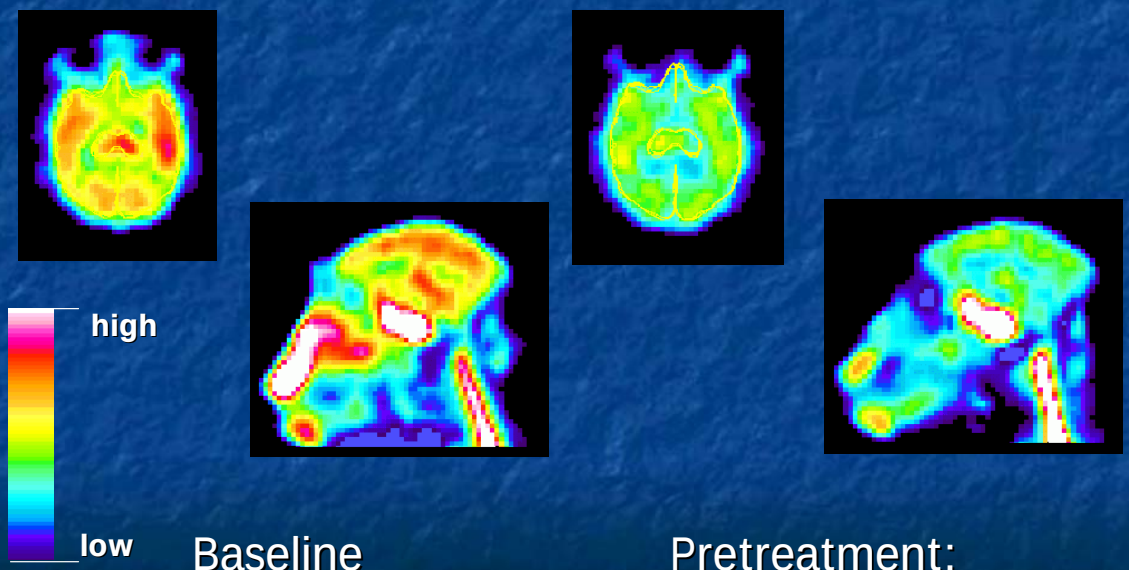


Ratios to striatum:
1.4 –1.6 at 140–180 min

Ratios to striatum:
1.0 –1.1 at end of study

Schou M. *et al., Synapse* (2004) 53, 57.

(*S,S*)-[^{18}F]FMeNER-D$_2$ in Cynomolgus monkey brain



Baseline

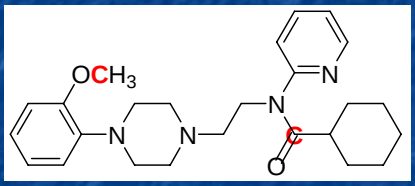
Pretreatment:
Desipramine (5 mg/kg)

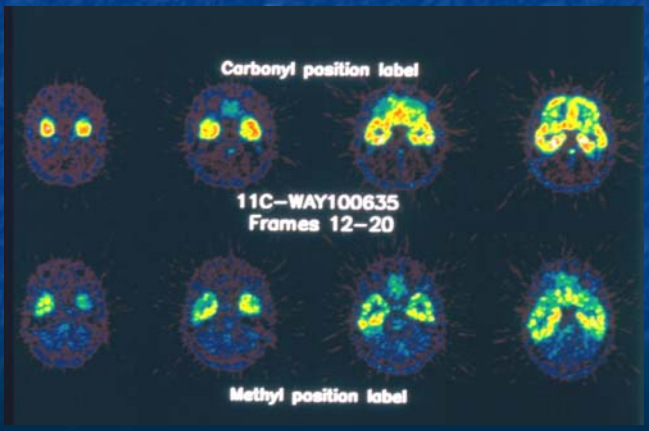
Metabolism: How should we avoid troublesome radiometabolites?

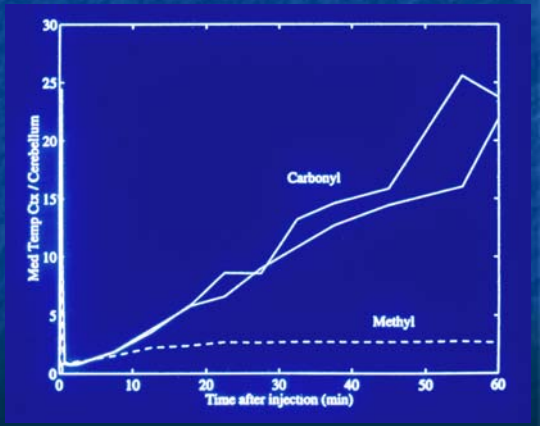
- Troublesome radiometabolite (TR) –a radioactive metabolite appearing in brain (or head) to interfere with PET imaging and quantitation of radioligand binding
- Predictable?
- Strategies to counter TR

  Ligand design; position of label; isotope effect; enzyme inhibition; mode of radioligand administration……

Comparison of [*methyl*-^{11}C]WAY and [*carbonyl*-^{11}C]WAY in human brain

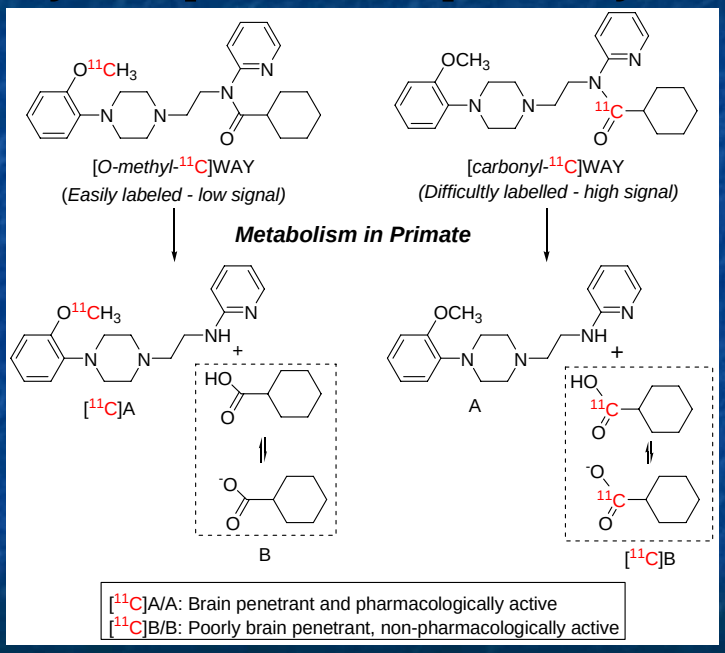






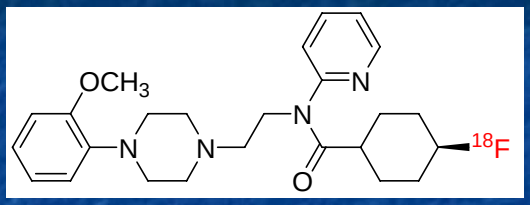
Pike V.W. *et al., Eur. J. Pharmacol.* (1996) 301, R5.

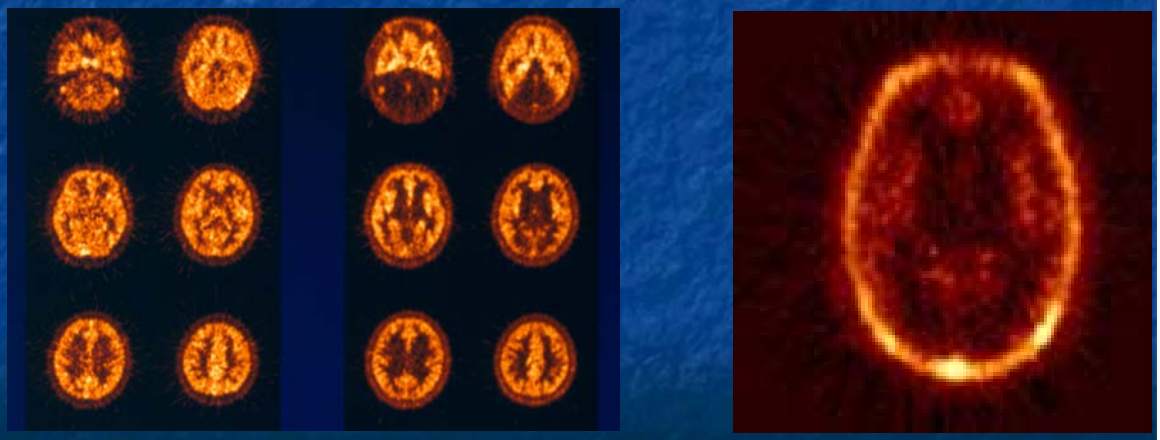
5-HT_{1A} Receptor radioligands: [*O-methyl*-^{11}C]WAY and [*carbonyl*-^{11}C]WAY



Osman S. *et al., Nucl. Med. Biol.* (1996) 23, 627-634 & (1998) 25, 215-223.

[^{18}F]FCWAY: Defluorinates in human



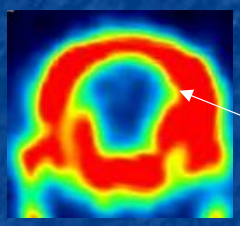


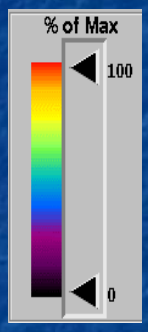
At 2–6 min **At 20–30 min** **At 2 h**

Courtesy of Carson R. *et al.* (formerly NIH)

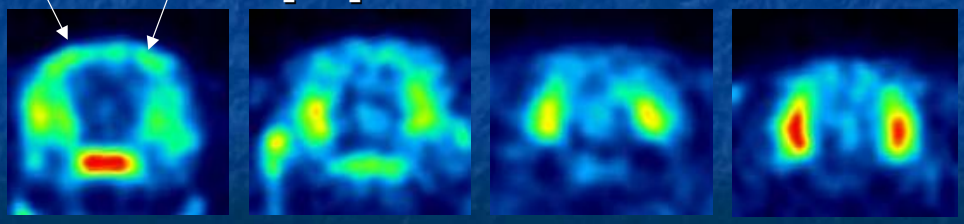
Miconazole inhibits [^{18}F]FCWAY defluorination & bone uptake in rat

[^{18}F]Fluoride ion





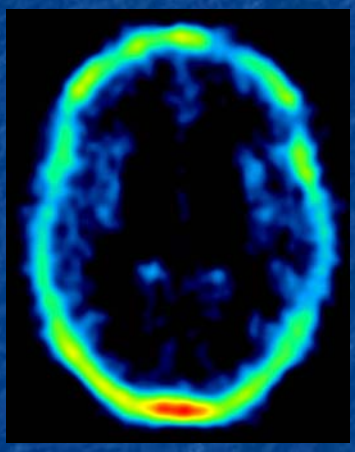
[^{18}F]FCWAY: Miconazole



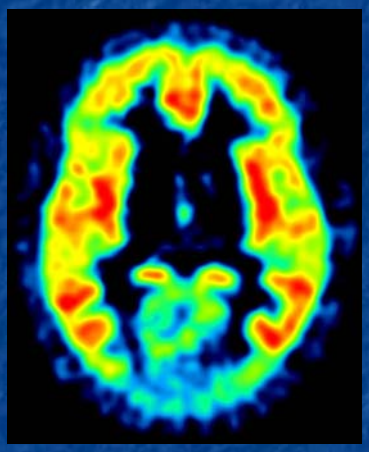
Baseline **15 mg/kg** **30 mg/kg** **60 mg/kg**

Tipre D. *et al. J. Nucl. Med.* (2006) 47, 345–353.

In human disulfiram decreases skull activity and increases brain uptake of [^{18}F]FCWAY



Baseline



Disulfiram

Images at 2 h in same subject. Disulfiram 500 mg PO prior night

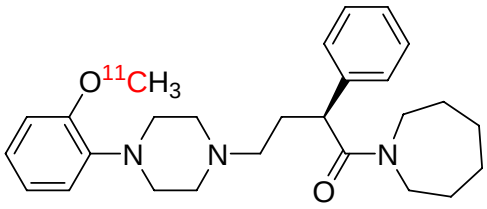
Ryu Y.H. *et al.* Submitted

Brain penetration: predictable?

- Traditional criteria: MW < 500; *LogP* 1.5–3.5; no formal charge
- *LogP* measured or determined from structure by one of various programs (*e.g.* Pallas 3.0). *LogD* (at pH 7.4 may also be calculated)

P = octanol-water partition coefficient

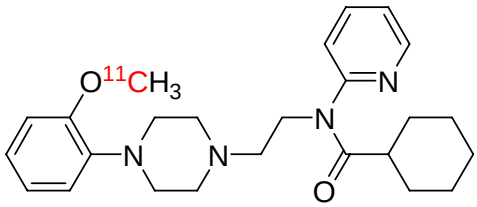
[*O-methyl*-^{11}C]RWAY:
metabolically resistant & easy to label



[*O-methyl*-^{11}C]RWAY

High affinity antagonist
Easily labeled
Resistant to metabolic cleavage?

c.f.

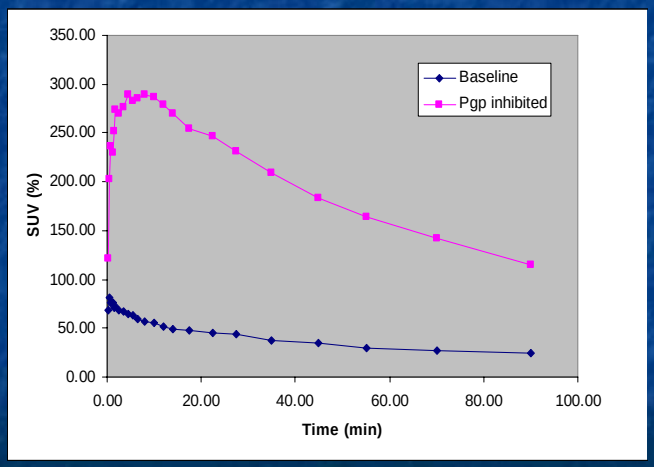


[*O-methyl*-^{11}C]WAY-100635

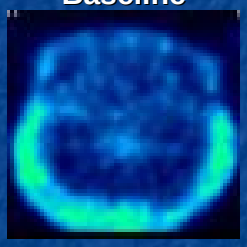
McCarron J.A. *et al., NeuroImage* (2006) 22 (Suppl. 2), T34-T35.
***Eur. J. Nucl. Med. & Mol. Imaging,* In press**

[^{11}C]RWAY in rat: P-gp inhibition increases brain uptake

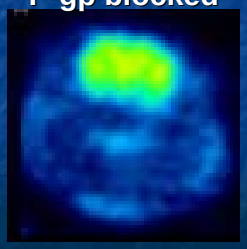
P-glycoprotein efflux pump: removes many drugs from brain



Baseline



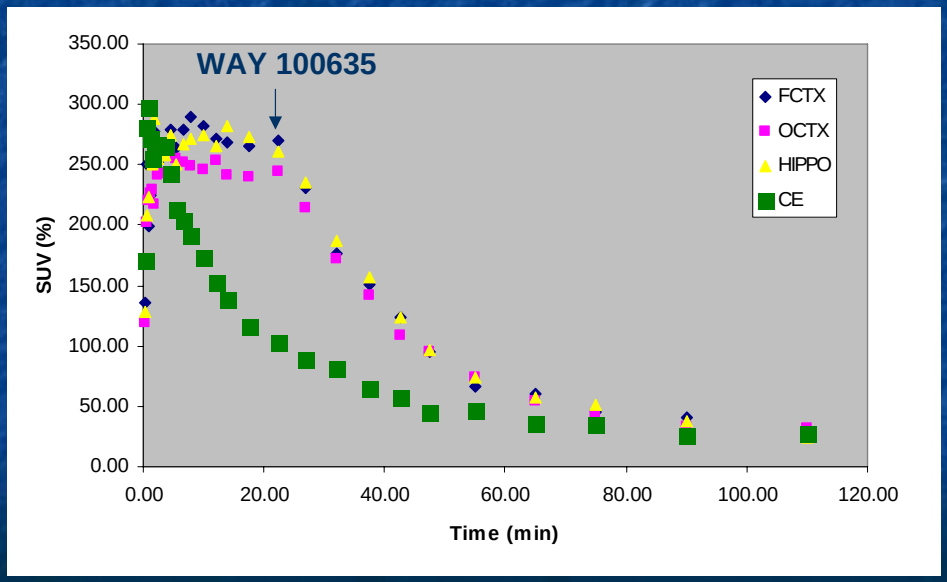
P-gp blocked



Block: Cyclosporin-A given 30 min before radioligand for P-gp inhibition

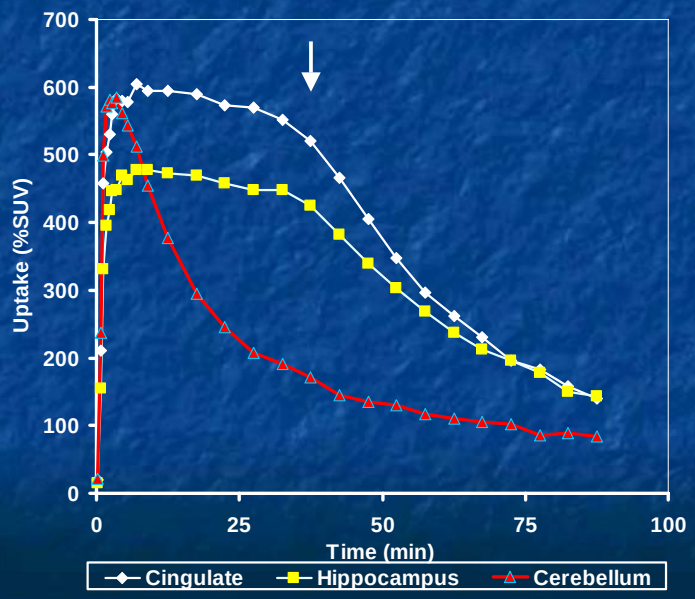
Liow J.-S. *et al.*, *Synapse* (2007) 61, 96-105

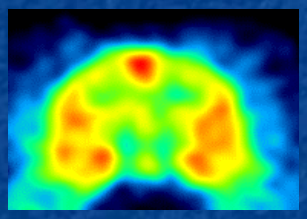
[^{11}C]RWAY in rat with P-gp inhibition



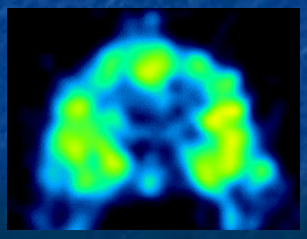
Liow J.-S. *et al.*, *Synapse* (2007) 61, 96-105

[^{11}C]RWAY in Monkey: displacement with WAY-100635





Before displacement



After displacement

Yasuno F. *et al. Synapse* (2006) 60, 510-520.

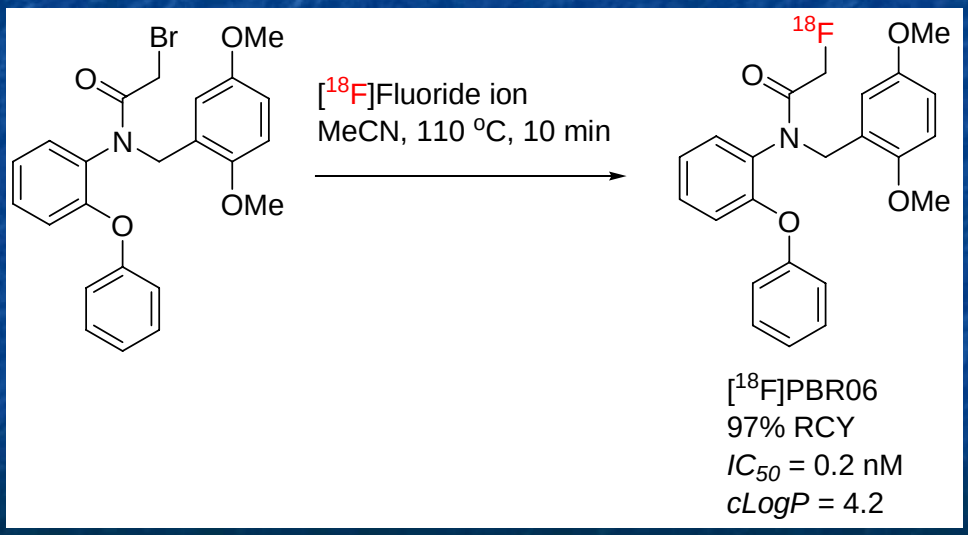
Amenability to labeling

- Ideally radioligand should be amenable to efficient labeling in one step and in high specific radioactivity
- [^{11}C]CO, [^{11}C]methyl iodide, [^{11}C]methyl triflate and [^{18}F]fluoride ion are especially useful

‘Peripheral benzodiazepine receptor’

- Brain: labels intracellular protein on mitochondria of microglia and astrocytes
- Marker for (neuro)inflammation
- Uses: stroke, MS, and Alzheimer’s disease
- First ligand [^{11}C]PK 11195 has low brain uptake and small signal

New ‘peripheral benzodiazepine receptor’ (PBR) radioligand: [^{18}F]PBR06



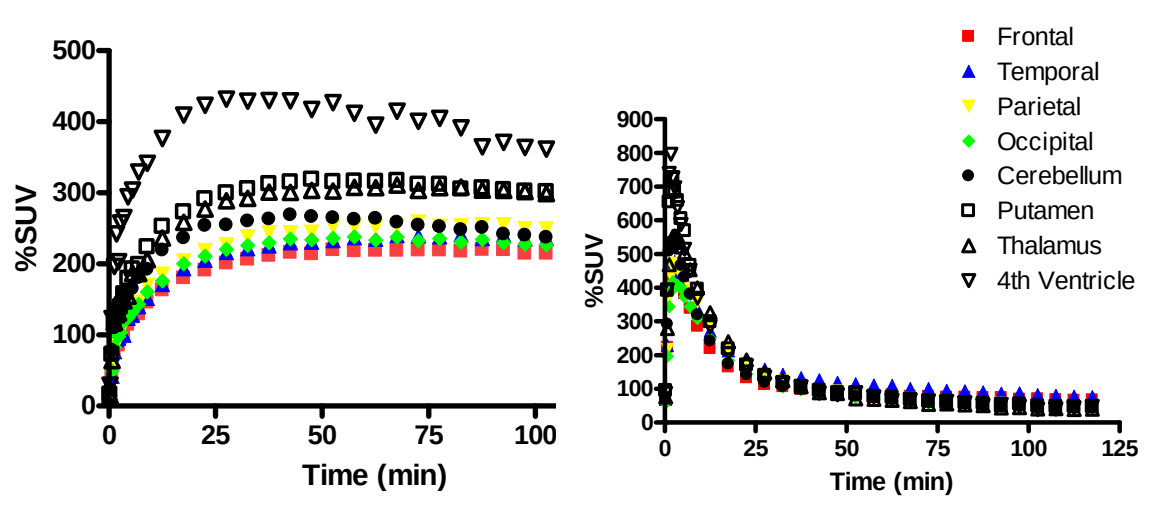
Briard E. *et al., J. Label. Compd. Radiopharm.* (2005) 48 (Suppl. 1), S4

[^{18}F]PBR06 in rhesus monkey

Baseline study TAC

Blocking study TAC

3 mg/kg DAA 1106 at 24 min before injection of [^{18}F]PBR06

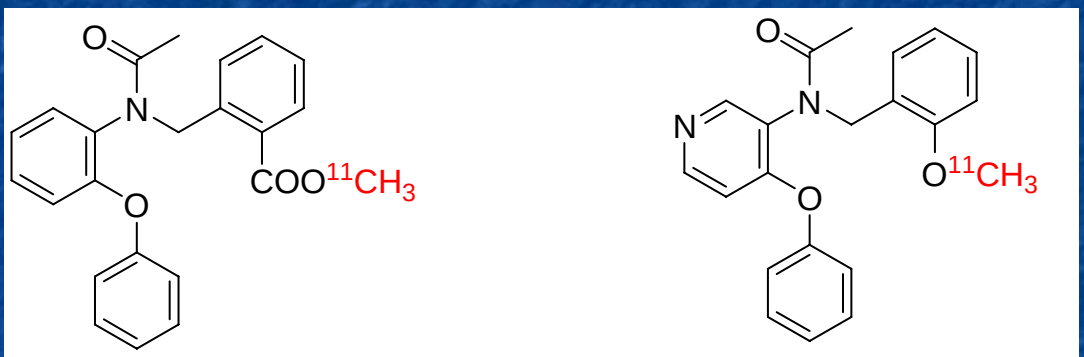


Injection dose: 5.80 mCi
SA: 587 mCi/µmol
Mass dose: 3.91 µg

Injection dose: 5.09 mCi
SA: 440 mCi/µmol
Mass dose: 4.58 µg

Imaizumi M. *et al.*, *Synapse* (2007)

New ^{11}C-labeled PBR radioligands



[^{11}C]PBR01
IC_{50} = 0.06 nM; *cLogP* = 4.2

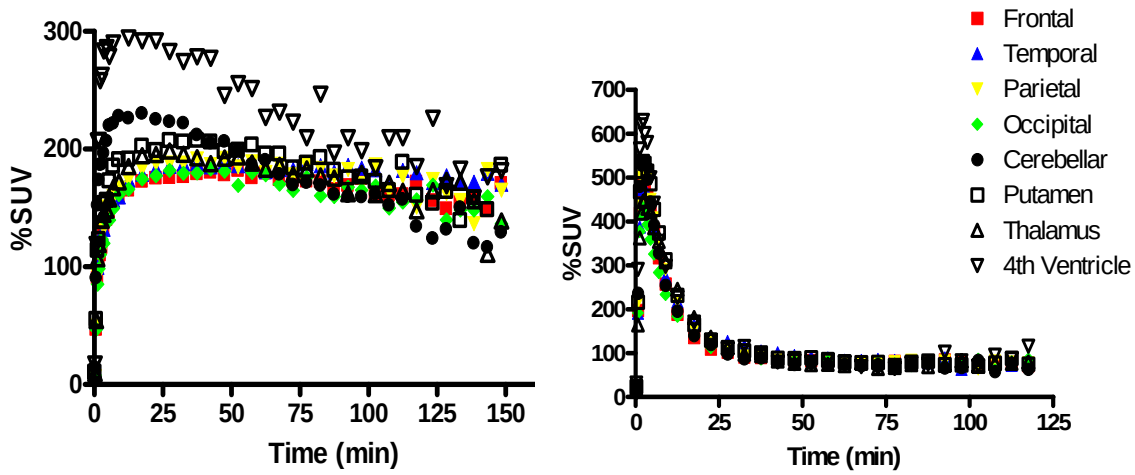
[^{11}C]PBR28
IC_{50} = 0.6 nM; *cLogP* = 3.0

Briard E. *et al., J. Label. Compd. Radiopharm.* (2005) 48 (Suppl. 1), S71

[^{11}C]PBR01 in monkey

Baseline study TAC

Blocking study TAC
PBR01 1 mg/kg i.v.



Injection dose: 3.71 mCi
SA: 557 mCi/µmol
Mass dose: 2.50 µg

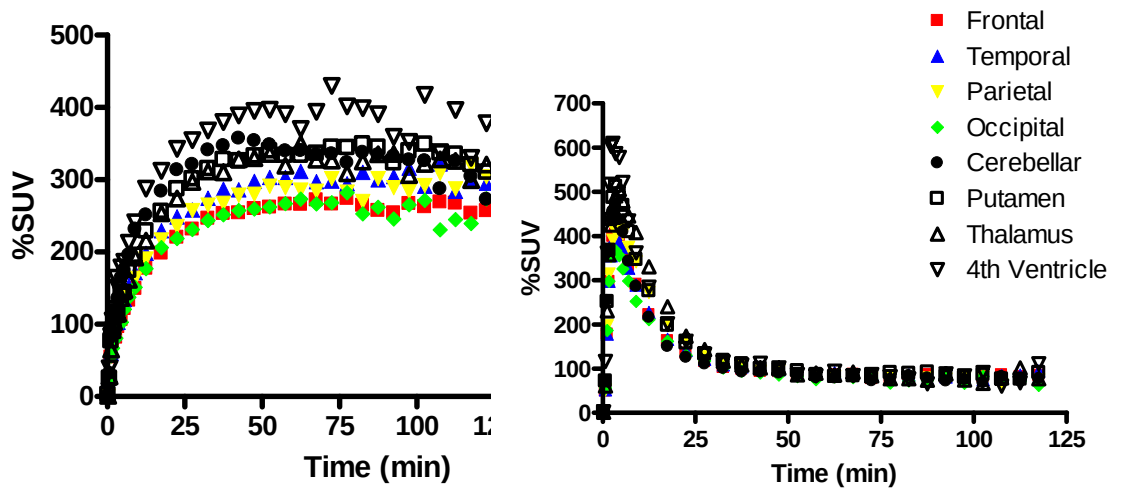
Injection dose: 2.84 mCi
SA: 2,972 mCi/µmol
Mass dose: 0.36 µg

Imaizumi M. *et al.*, *Synapse* (2007)

[^{11}C]PBR28 in monkey

Baseline study TAC

Blocking study TAC
Cold PBR28 3 mg/kg i.v.



Injection dose: 4.23 mCi
SA: 1,122 mCi/µmol
Mass dose: 1.31 µg

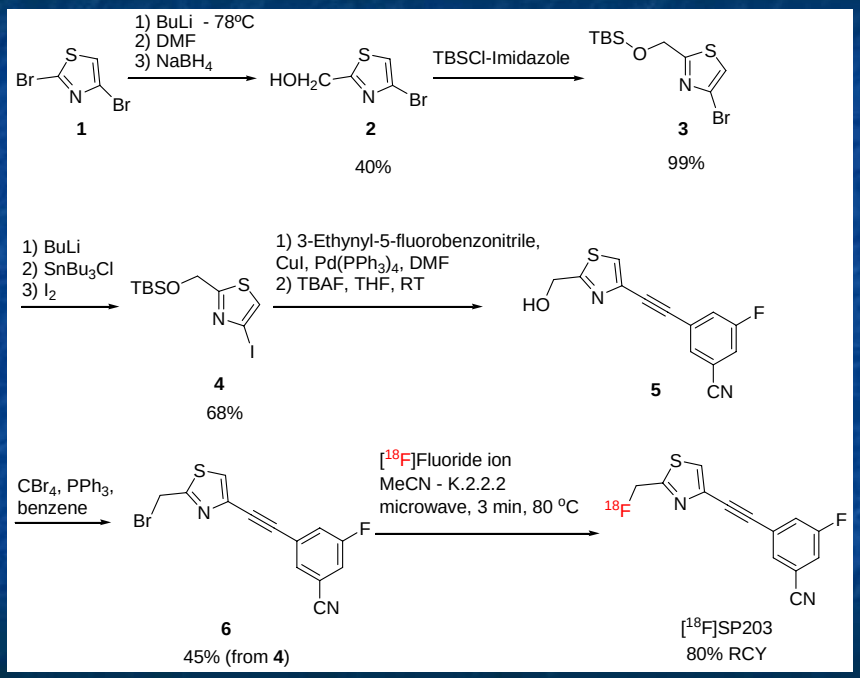
Injection dose: 5.47 mCi
SA: 1,260 mCi/µmol
Mass dose: 1.51 µg

Briard E. *et al.*, In preparation

Metabotropic Glutamate Receptor: mGluR5

- Glutamate: primary excitatory neurotransmitter
  - Metabotropic: G-protein coupled
  - Ionotropic: fast ion channels
- mGluR5: abundant in striatum, hippocampus, and amygdala; low in cerebellum
- mGluR5: Pharma targets for anxiety & psychosis

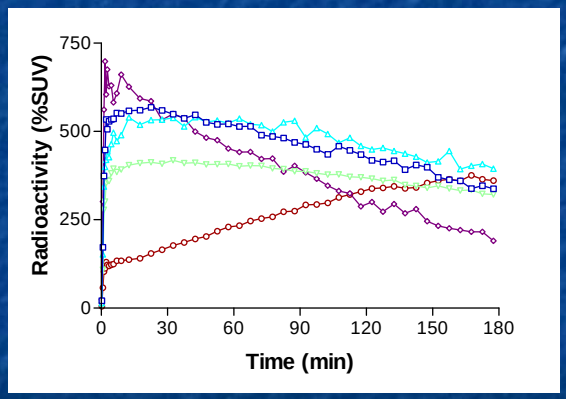
New mGluR5 radioligand: [^{18}F]SP203



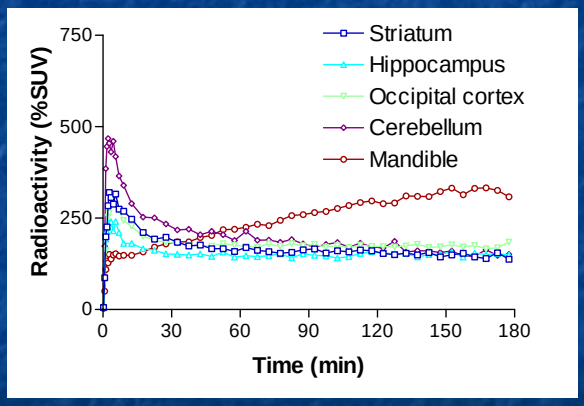
Siméon F. *et al.*, *Abstr. Am. Chem. Soc.* (2005)
***J. Med. Chem.* In press**

[^{18}F]SP203: in Rhesus monkey brain

Baseline



Preblock

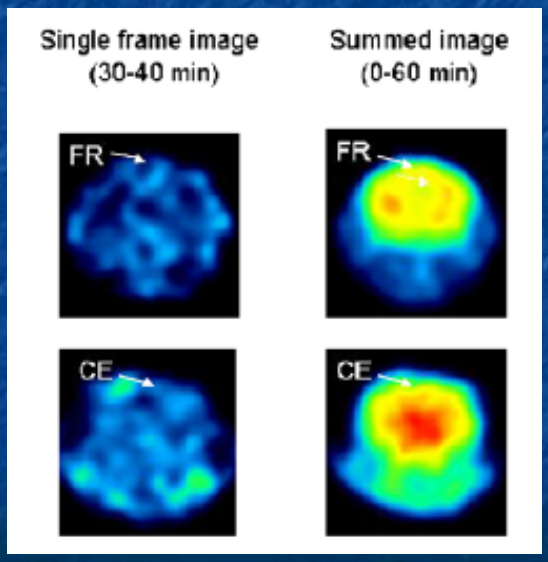


Siméon F. *et al.*, *J. Med. Chem.* In press

Characterized binding site?

- Species differences in nature of binding site may occur
- Multiple binding sites may occur in same target protein
- Striking example; binding site for β-amyloid plaque radioligands *e.g.* [^{11}C]PIB

[^{11}C]PIB in mouse Tg2756 model of Alzheimer's disease



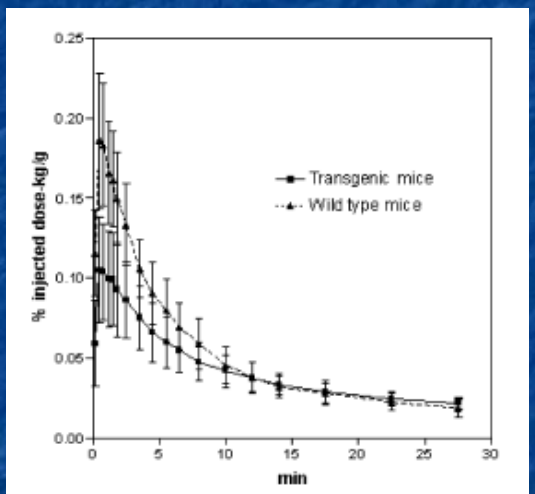
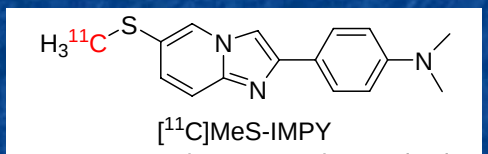


Fig. 3. Representative averaged time–activity curves (±SD) of [^{11}C] 6-OH-BTA-1 in the frontal cortex

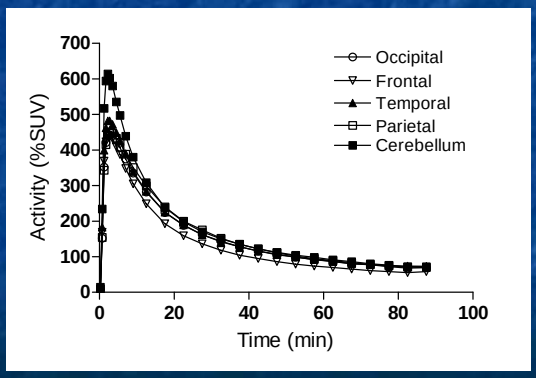
Toyama H. *et al., Eur. J. Nucl. Med.* (2005) 32, 593.

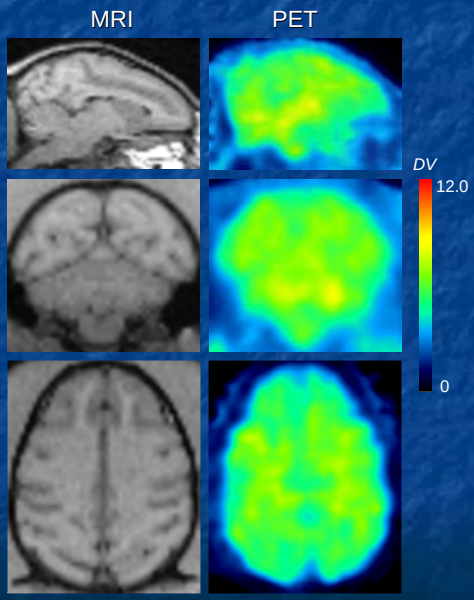
[^{11}C]MeSIMPY in rhesus monkey – high brain uptake and fast washout



[^{11}C]MeS-IMPY

K_I = 7.93 nM at human Aβ plaques *in vitro*





Cai L. *et al.*, In preparation; Seneca N. *et al.*, In preparation

Radioligand Discovery: Craft or Science?

- Some parameters predictable and/or partly controllable *e.g.*

  K_D,

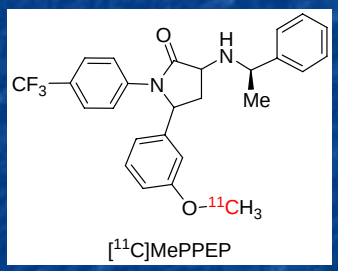
  LogP
- Others not *e.g.*

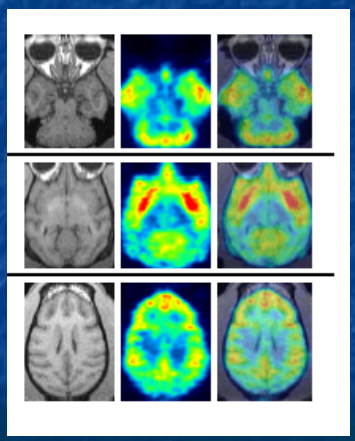
  brain penetration,

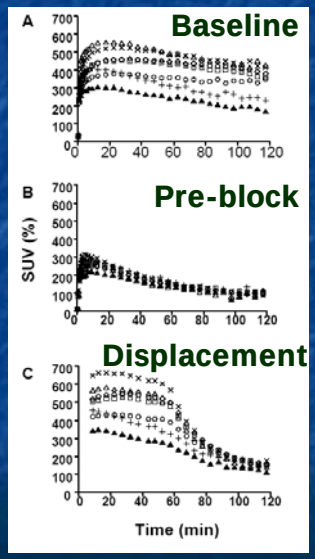
  effects of metabolism,

  inter-species differences...

[^{11}C]MePPEP images brain CB_1 receptors in monkey







Yasuno F. *et al.*, *Neuropsychopharmacology* (2007) 1-11.

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