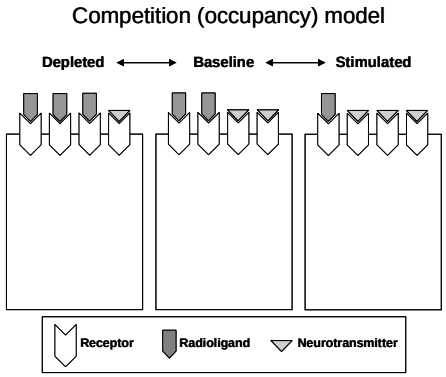
How to establish a new radiotracer

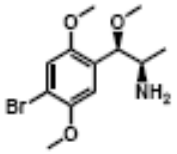
- Target identified
  - Enough binding sites?
- Candidates
  - Suitable affinity, selectivity, lipophilicity?
  - Suitable chemical structure?
  - Agonist, antagonist properties?
- Test
  - autoradiography, animal PET
- Metabolism
  - No lipophilic metabolites, not too rapid
- Protein binding of compound
- Uptake in target regions
  - Can binding be specifically blocked
- Quantification issues
  - 1TC, 2TC, linear methods, reference tissue methods
- Mass dose issues

Why a 5-HT_{2A} receptor agonist PET tracer?

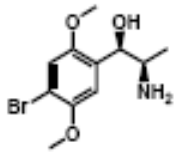
- Dopamine D_2 receptor agonist radiotracers are superior to antagonist radiotracers for measuring dopamine release *(Narendran et al. 2010, 2004; Cumming et al. 2002)*.
- 5-HT_{2A} receptor antagonist PET tracers are generally not displaceable by elevated levels of 5-HT *(Pinborg et al. 2004, Paterson et al. 2010)*.
- 5-HT_{2A} agonist PET tracers are hypothesised to be more prone to displacement by endogenous 5-HT.



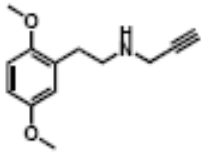
Some Medicinal Chemistry 5-HT_{2A} Receptor Compounds



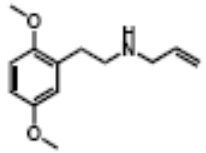
Cimbi 1



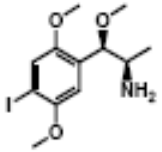
Cimbi 2



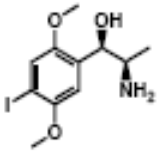
Cimbi 9



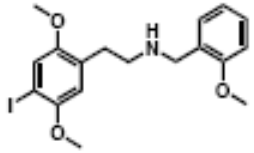
Cimbi 10



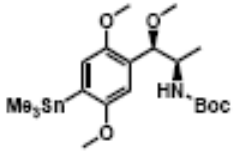
Cimbi 3



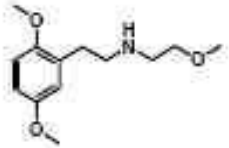
Cimbi 4



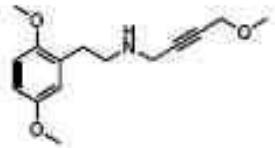
Cimbi 5



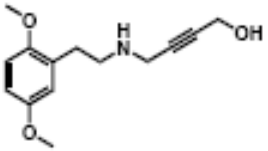
Cimbi 6



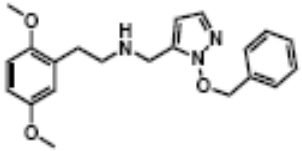
Cimbi 11



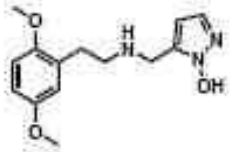
Cimbi 12



Cimbi 7



Cimbi 8



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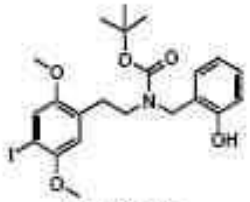
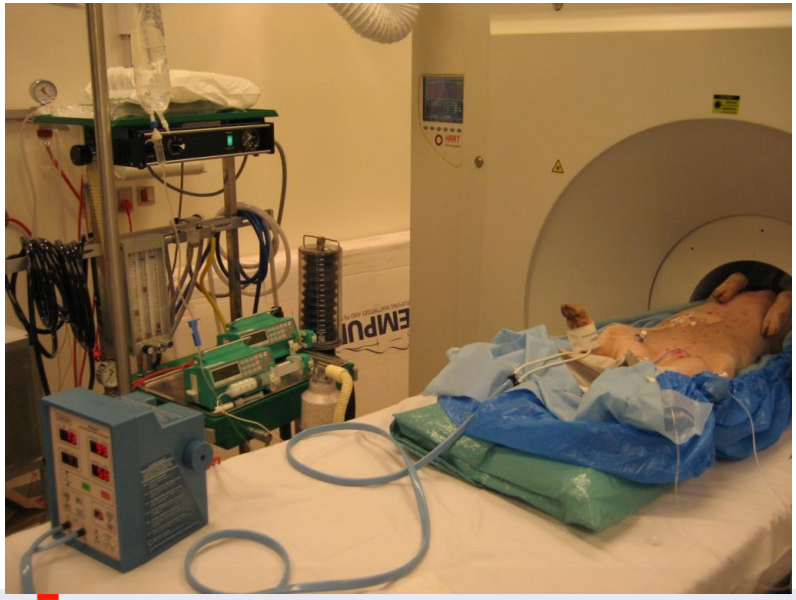
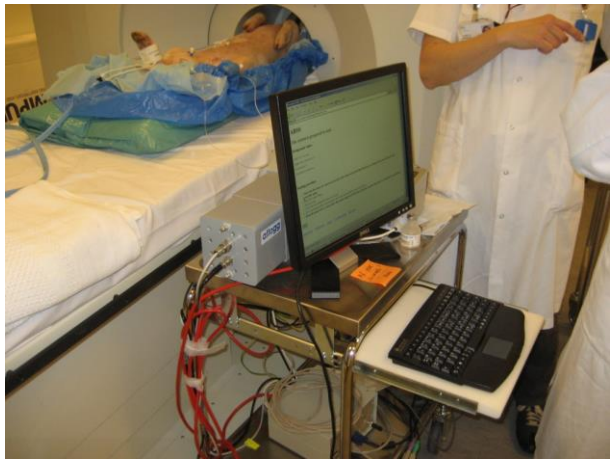


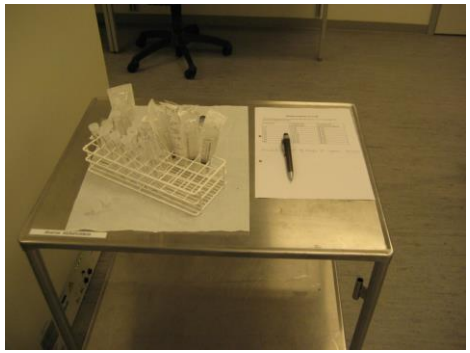
Cimbi 14

PET Methods: In pigs

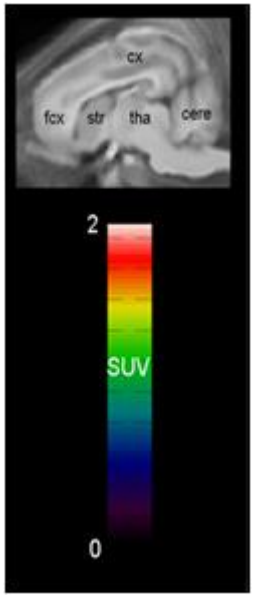
- Seven Danish Landrace Pigs (Weight 17.3±0.76 kg) were used in this study
- Anaesthesia: i.v. propofol (1 ml/kg*hour)
- Venous access through the milk vein, [^{11}C]Cimbi-5 given by IV bolus injections (mean injected dose: 238 MBq, mean specific activity: 71 GBq/µmol, and mean injected mass: 2.15 µg)
- Monitoring blood pressure, heart rate, and temperature during the scans
- Catheter in the femoral artery: Input using an autosampler, blood and plasma sampling manually

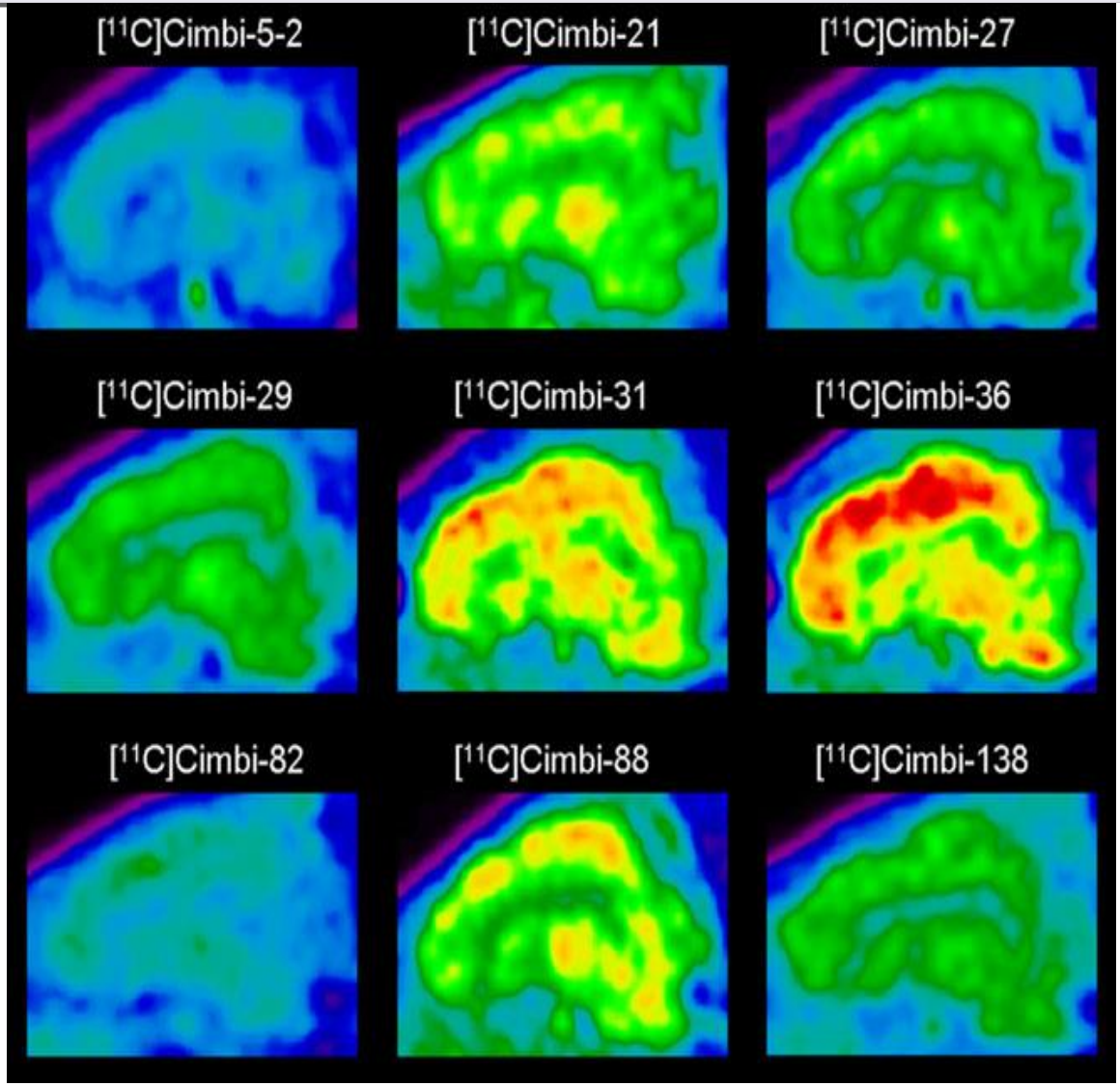




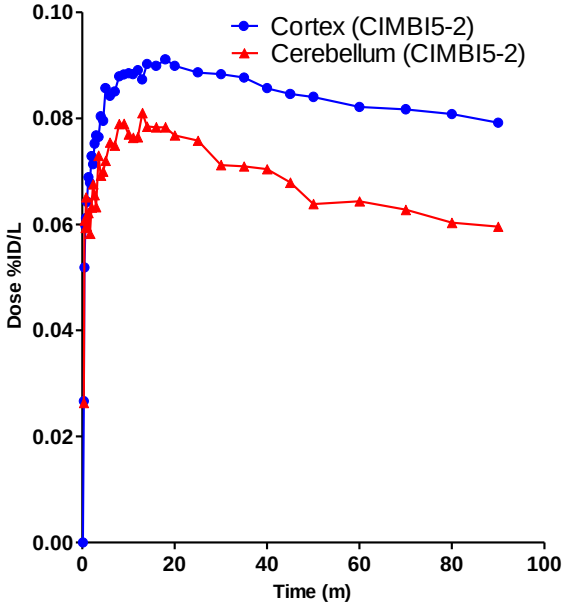


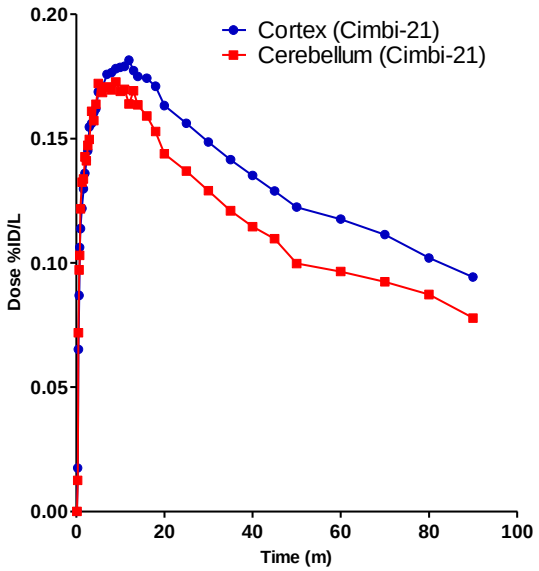
Nine ^{11}C-labelled 5-HT$_{2A}$ receptor agonists

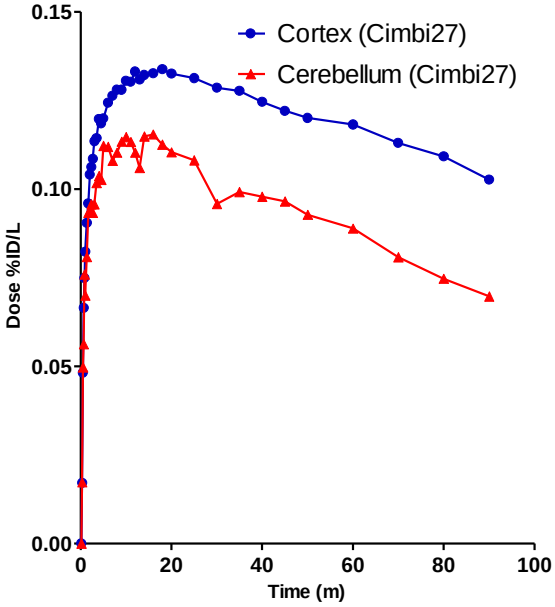


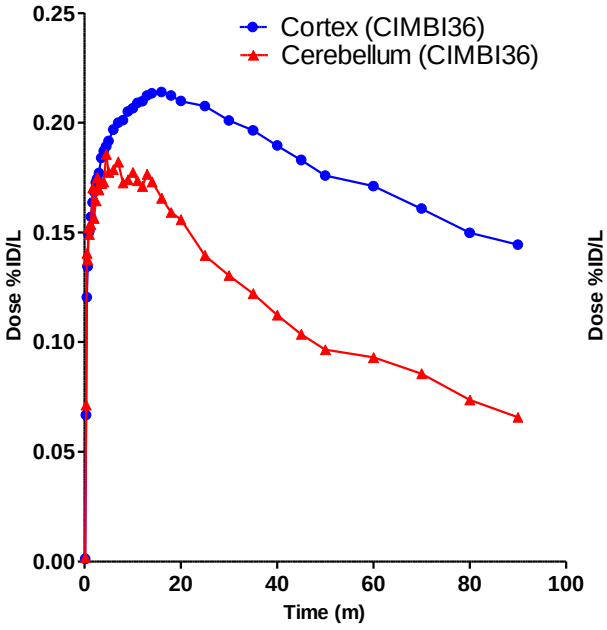


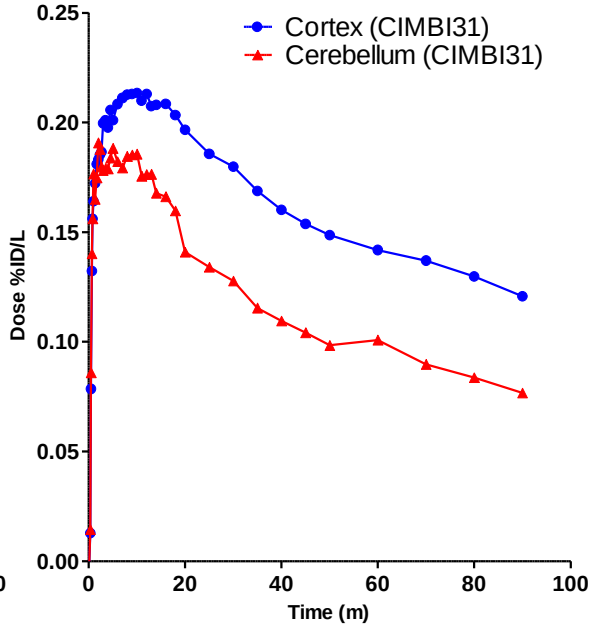
Ettrup et al, Eur J Nucl Med Mol Imaging, 2011

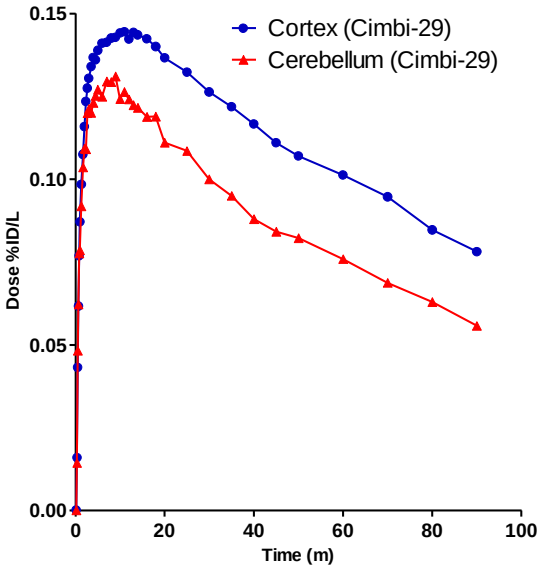

Cortex (CIMBI5-2)
Cerebellum (CIMBI5-2)
Dose %ID/L
Time (m)
0.00
0.02
0.04
0.06
0.08
0.10
0 20 40 60 80 100


Cortex (Cimbi-21)
Cerebellum (Cimbi-21)
Dose %ID/L
Time (m)
0.00
0.05
0.10
0.15
0.20
0 20 40 60 80 100

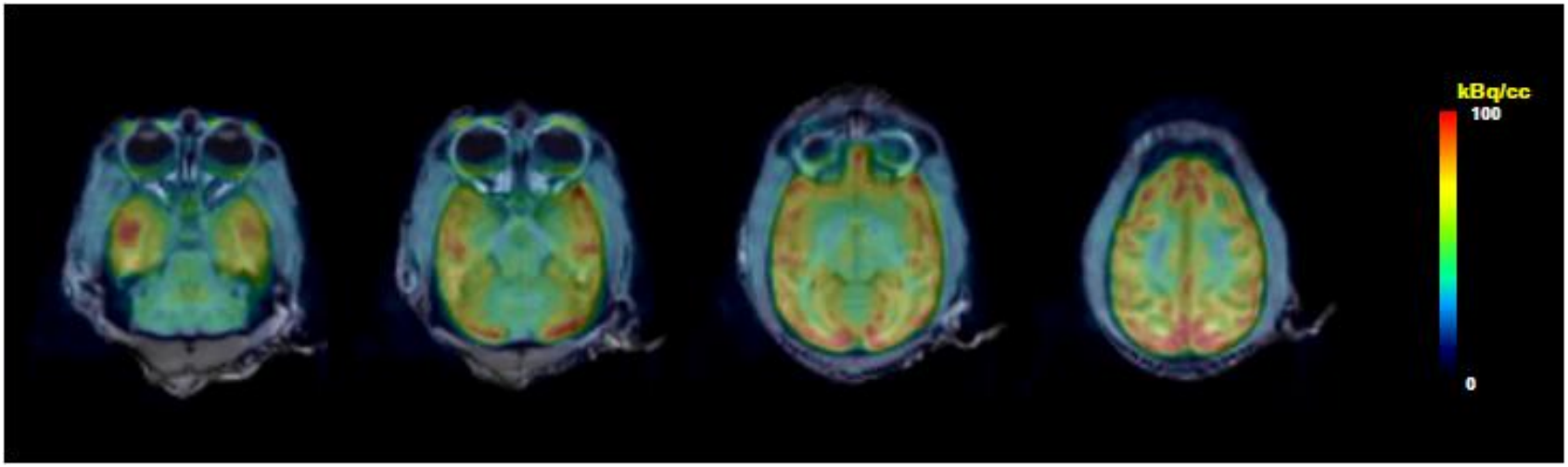

Cortex (Cimbi27)
Cerebellum (Cimbi27)
Dose %ID/L
Time (m)
0.00
0.05
0.10
0.15
0 20 40 60 80 100

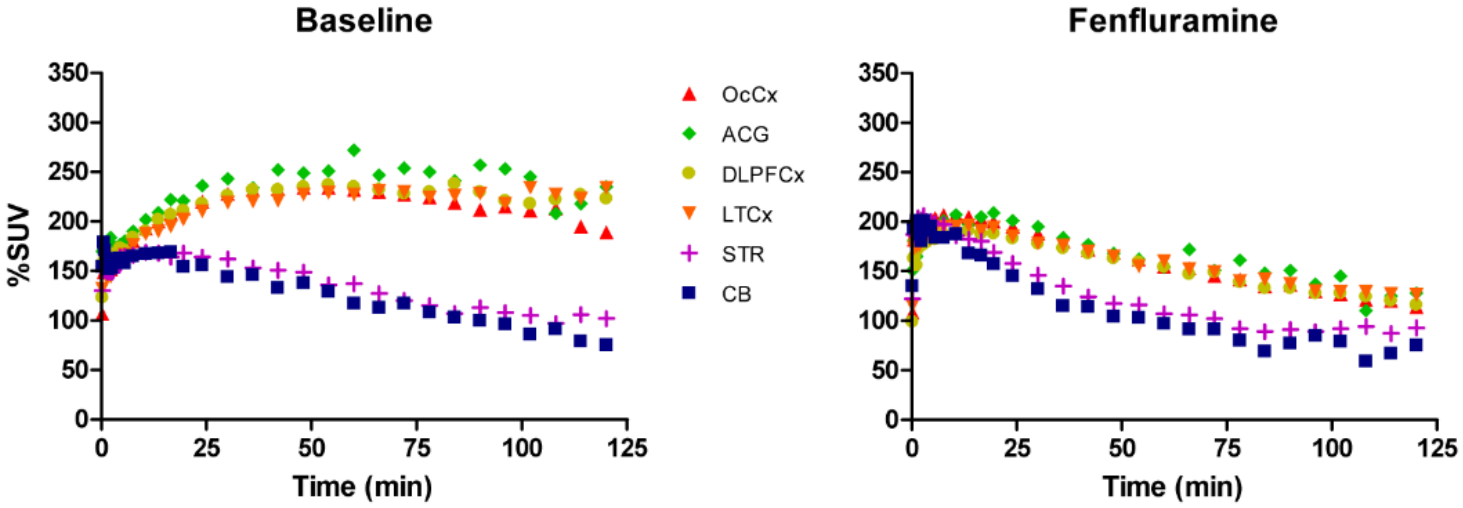

Cortex (CIMBI36)
Cerebellum (CIMBI36)
Dose %ID/L
Time (m)
0.00
0.05
0.10
0.15
0.20
0.25
0 20 40 60 80 100


Cortex (CIMBI31)
Cerebellum (CIMBI31)
Dose %ID/L
Time (m)
0.00
0.05
0.10
0.15
0.20
0.25
0 20 40 60 80 100


Cortex (Cimbi-29)
Cerebellum (Cimbi-29)
Dose %ID/L
Time (m)
0.00
0.05
0.10
0.15
0 20 40 60 80 100

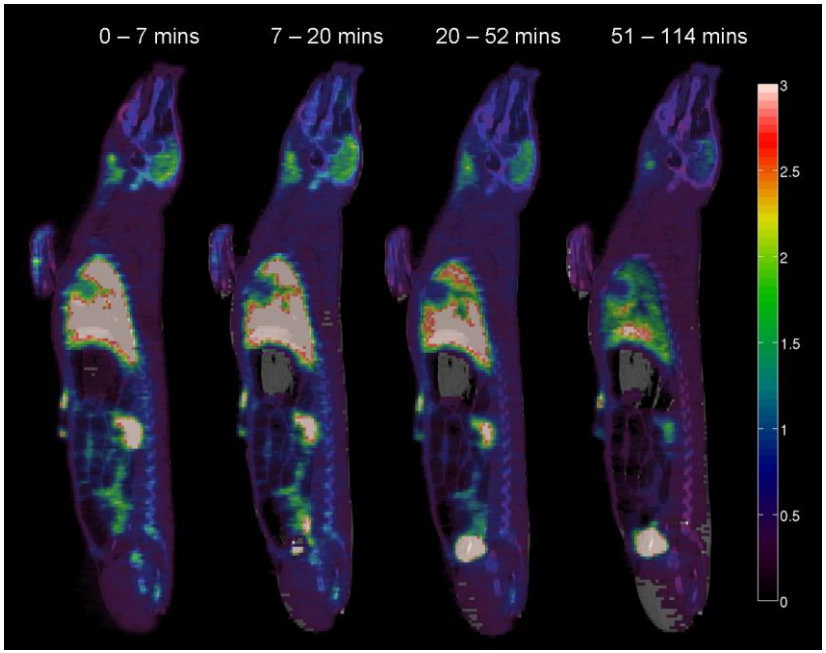
[^{11}C]Cimbi-36 is sensitive to fenfluramine

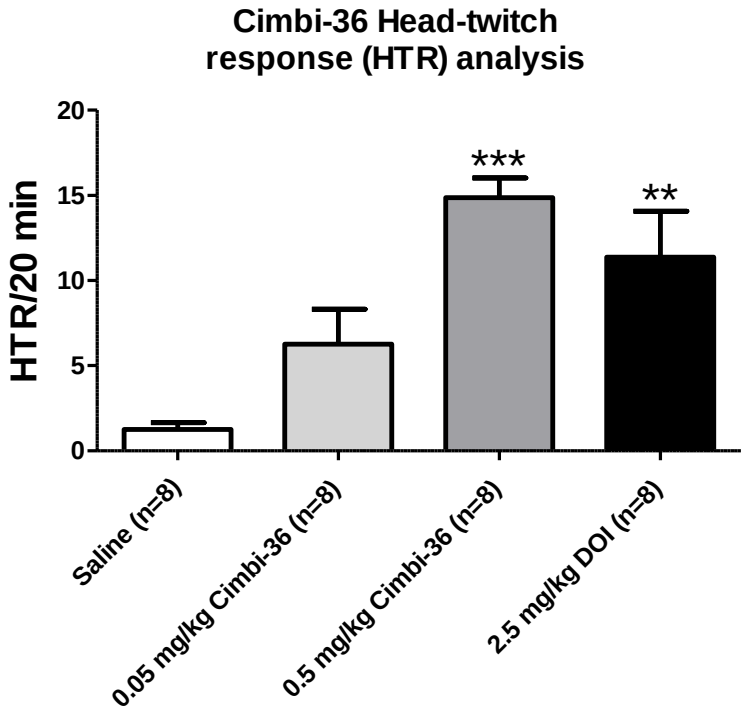




Finnema et al

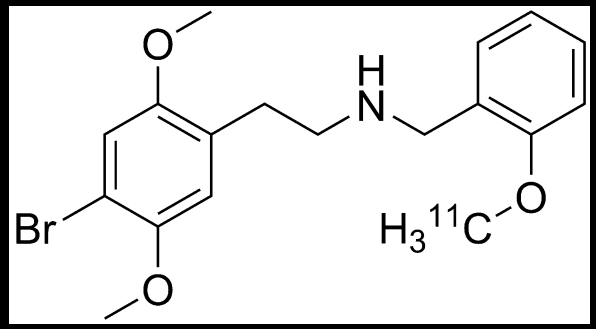
Safety and Dosimetry of [^{11}C]Cimbi-36

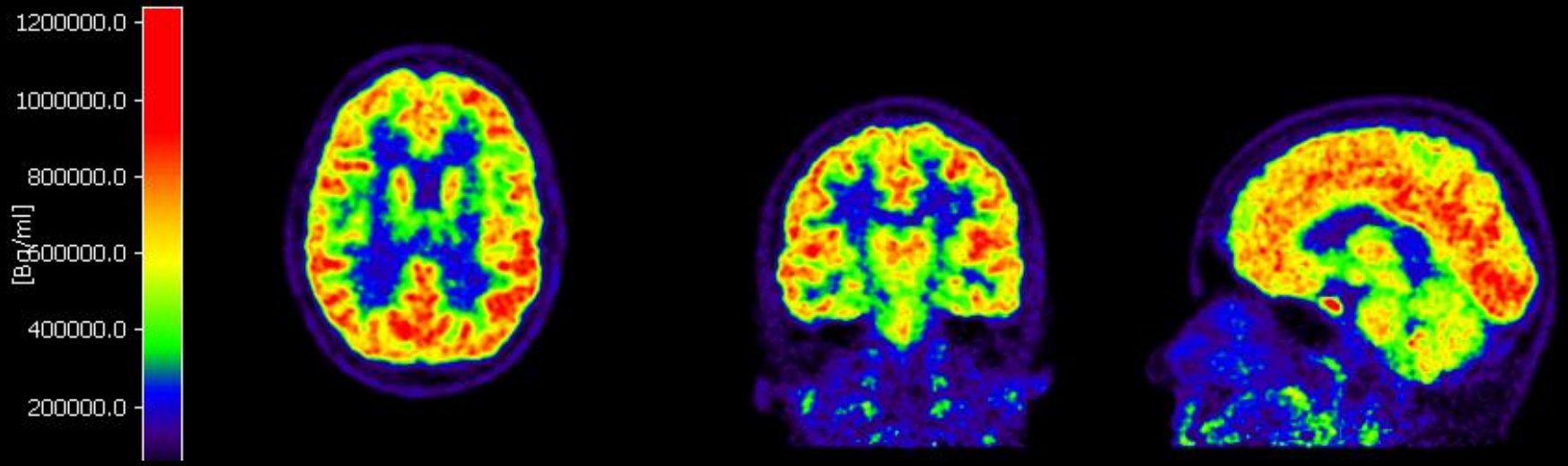




Ettrup et al, Mol Imaging Biol, 2013

[^{11}C]Cimbi-36 Brain Scan - First Time in Humans





PET- and Cyclotron Unit, RH, 2012

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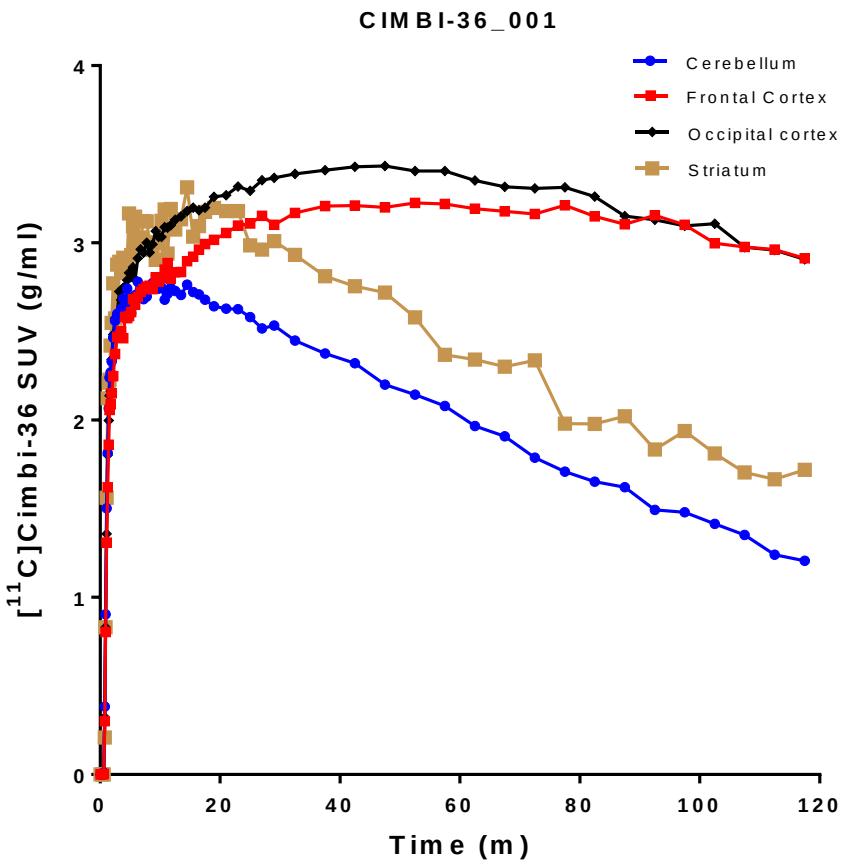
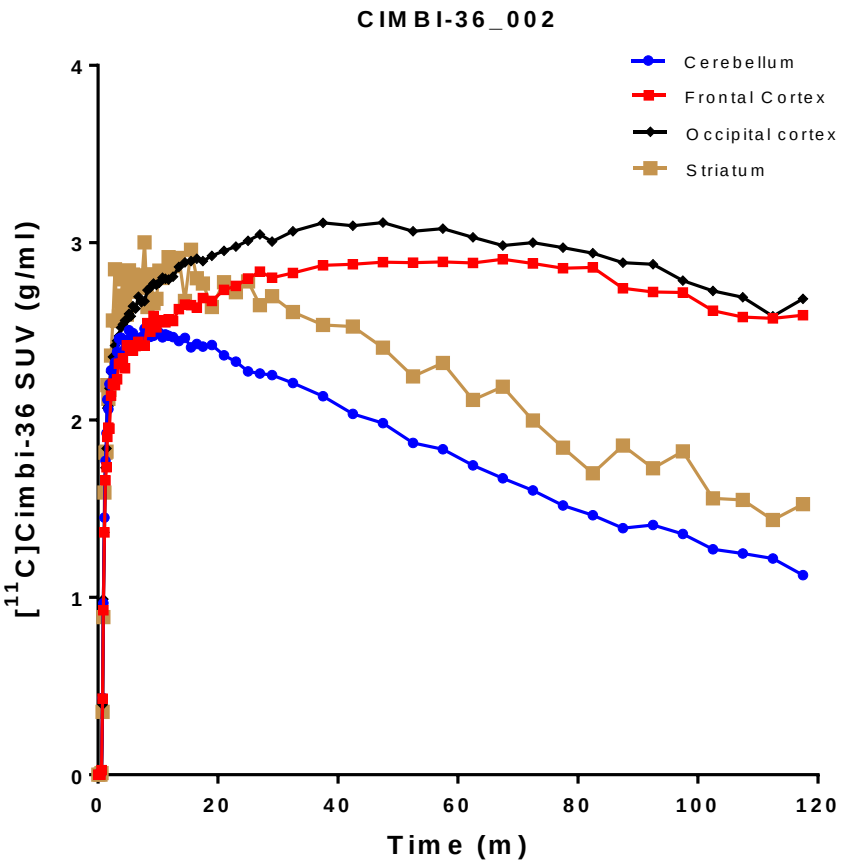
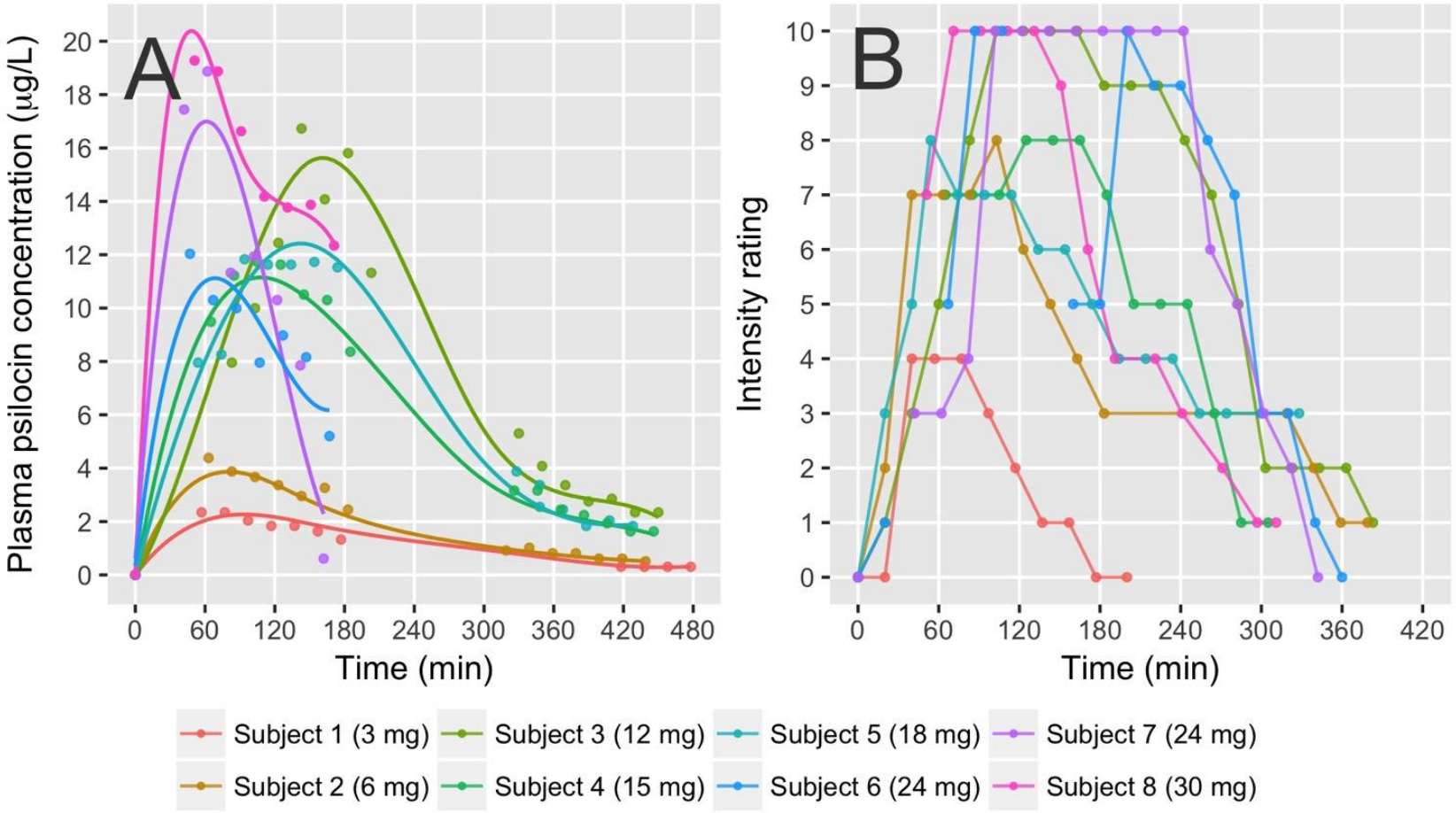
First Clinical [^{11}C]Cimbi-36 TACs





First subject was rescanned with [^{11}C]Cimbi-36 one hour after the first scan w/o any intervention between the scans (test-retest TACs are shown).

5-HT$_{2A}$R stimulation: Drug levels and subjective effects



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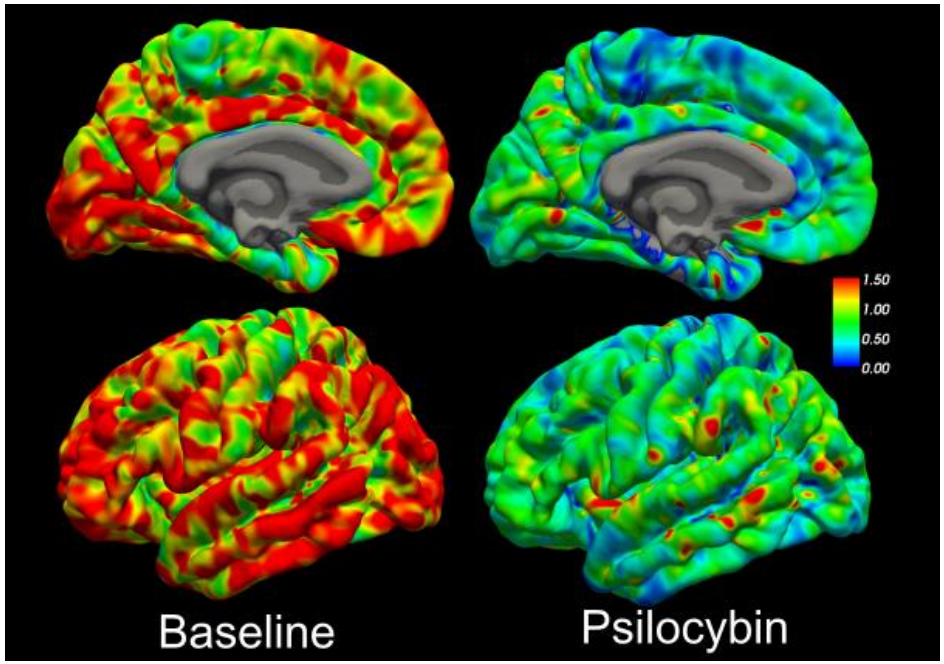
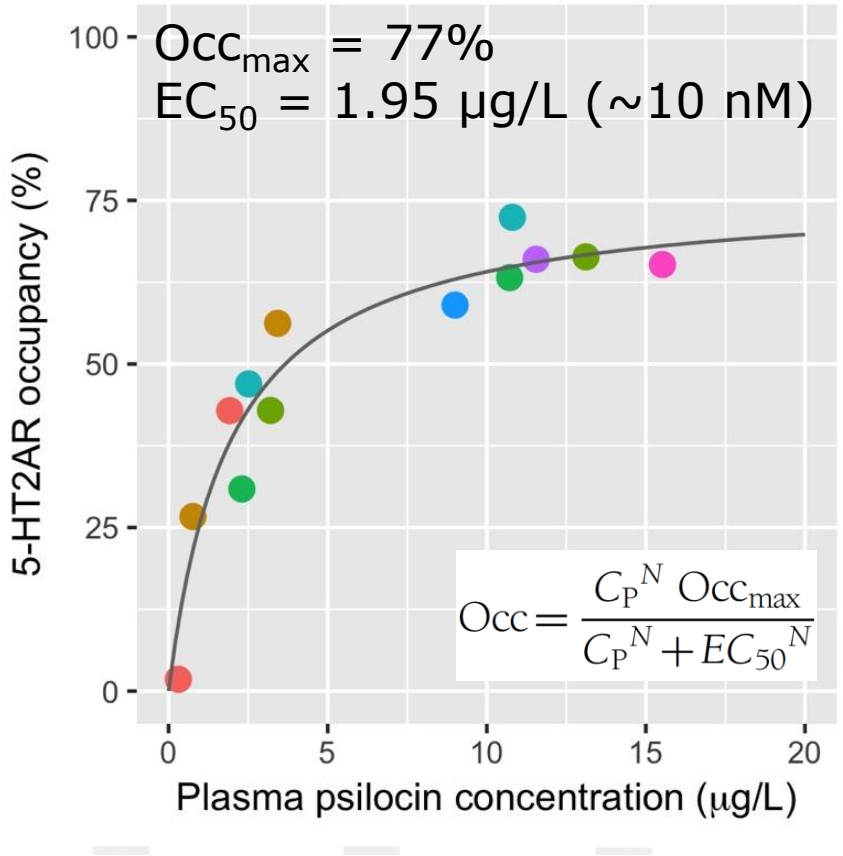
Madsen et al., *Neuropsychopharmacol 2019*

5-HT_{2A}R occupancy with [^{11}C]Cimbi-36 PET





Madsen et al., *Neuropsychopharmacol 2019*

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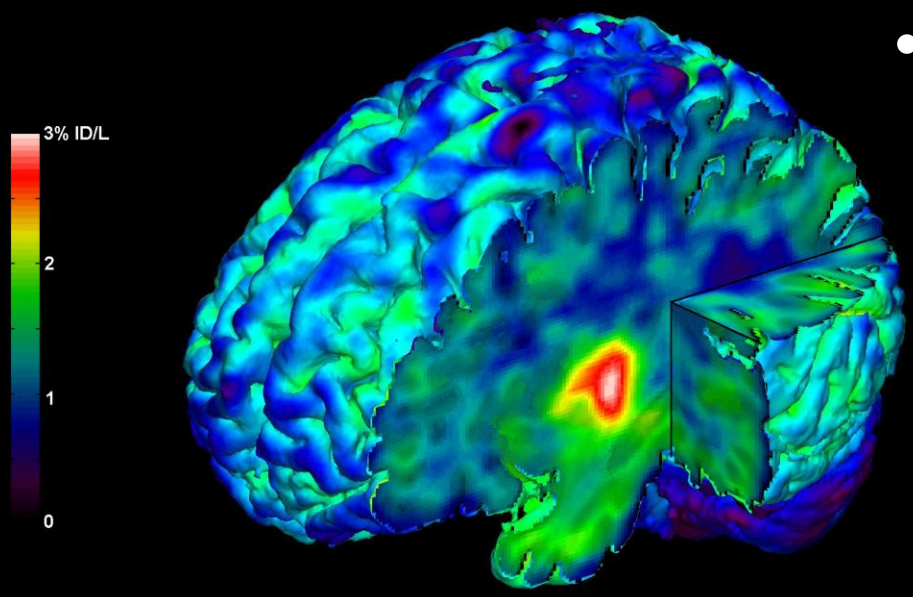
Center for integrated molecular brain imaging

5-HT_4-receptors

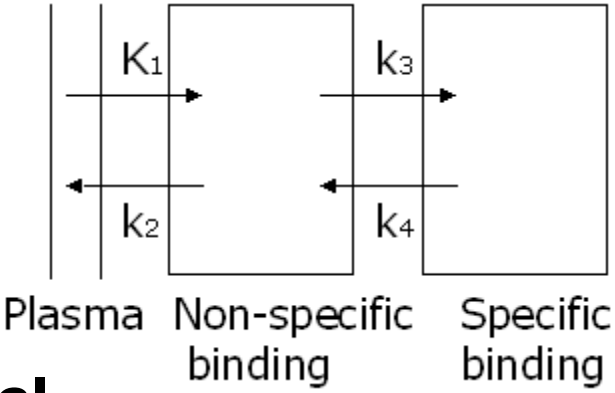


- Subgroup of serotonin receptors
- Located primarily in
  - caudate nucleus (32 pCi/mm^2)
  - putamen (26 pCi/mm^2)
  - hippocampus
    - CA1 (30 pCi/mm^2)
    - Dentate gyrus (17 pCi/mm^2)
  - cortical (12-20 pCi/mm^2)
  - Cerebellum (<1 pCi/mm^2)
- Clinical relevance:
  - Alzheimer's Disease
    - Enhance learning and memory
    - May diminish the deposition of pathological amyloid plaques

How do we quantitate the 5-HT_4 receptors?

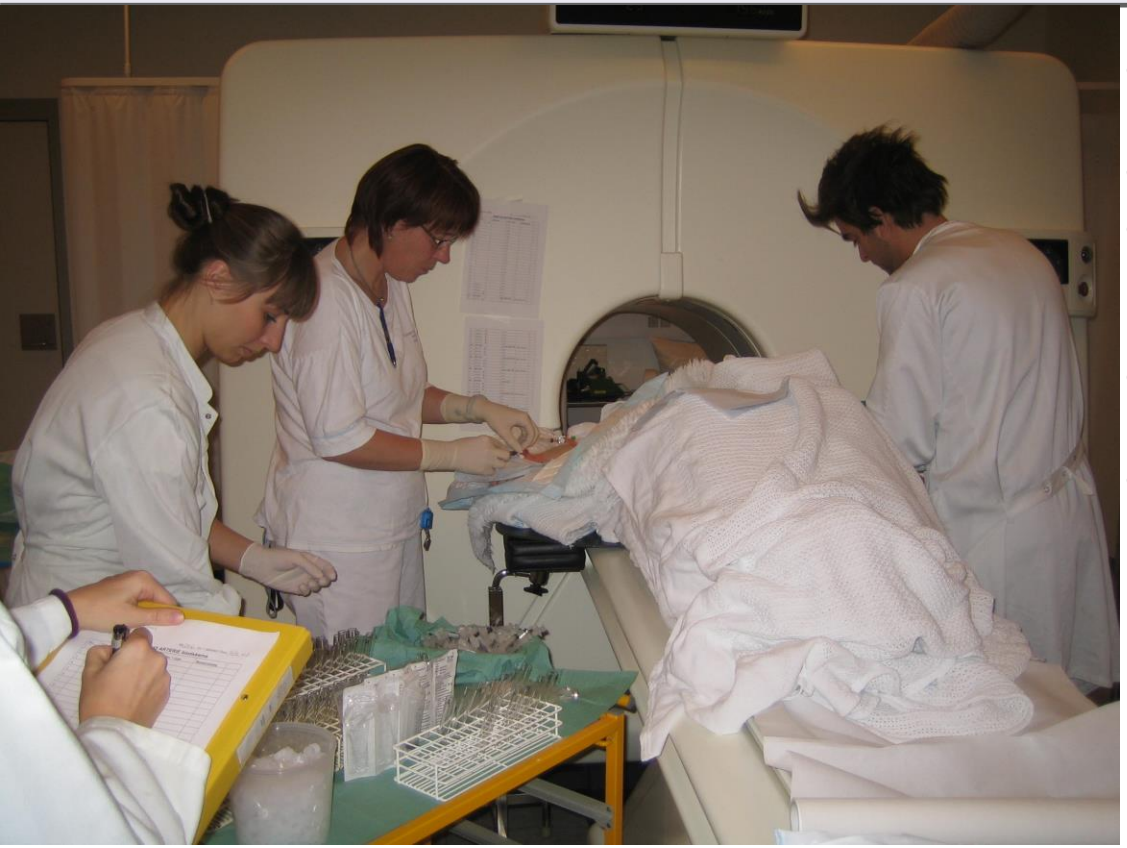
- New PET tracer: ^{11}C-SB207145 from GlaxoSmithKline

  –The ***Gold Standard*** for quantification is the use of a **compartment model** with arterial input with metabolite correction
    - Only few assumptions
    - Invasive with arterial cannulation
    - Noisy



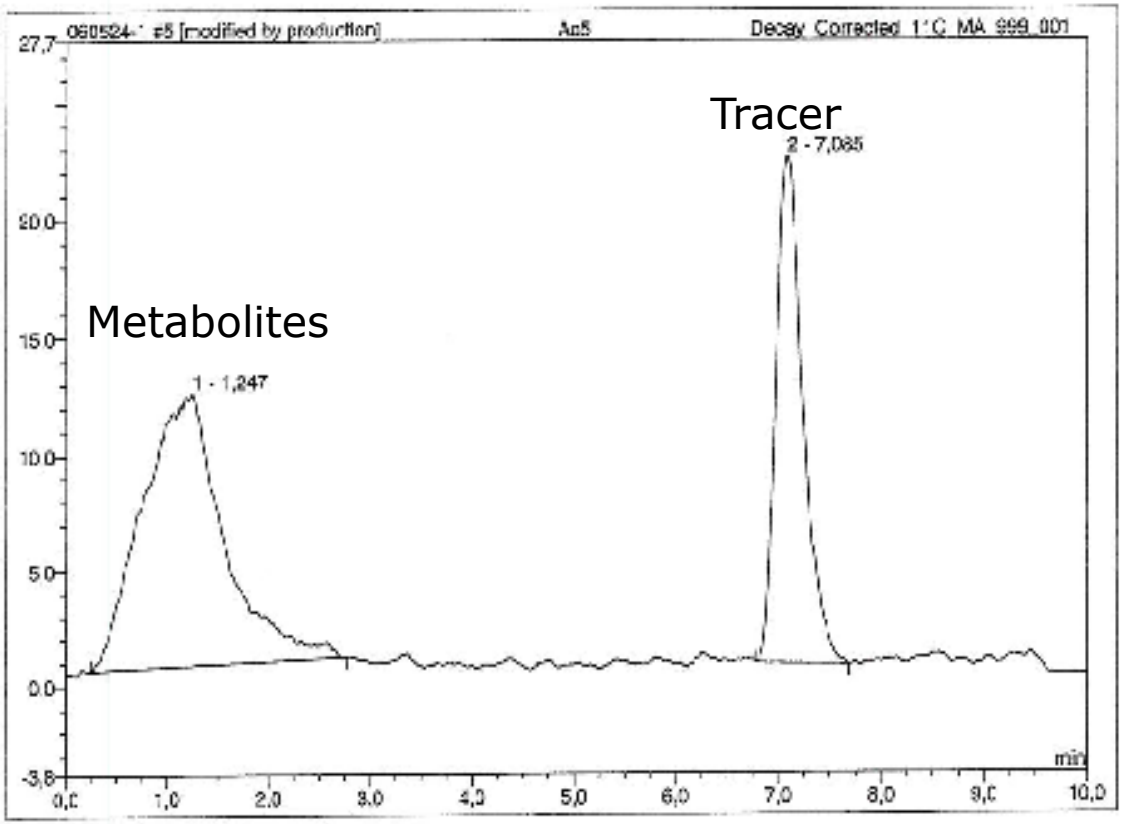
  –**Simplified reference tissue model**
    - Based on assumptions
    - Non-invasive
    - Needs validation

Set-up



- Nine healthy subjects - 6 were rescanned the same day
- Arterial cannulation
- 40-50 manually drawn blood samples during the scan for radioactivity measurement
- 4-7 samples for metabolite measurements
- 2-hour scan in 18 ring GE-advance scanner
  - Reconstructed using filtered back projection
  - Automatic delineation of regions of interest (Svarer et al. Neuroimage 24 (2005): 969-79)
  - Partial volume correction (Müller-Gartner et al. J.Cereb.Blood Flow Metab (1992):571-83

Metabolite measurements



Fraction of unmetabolized

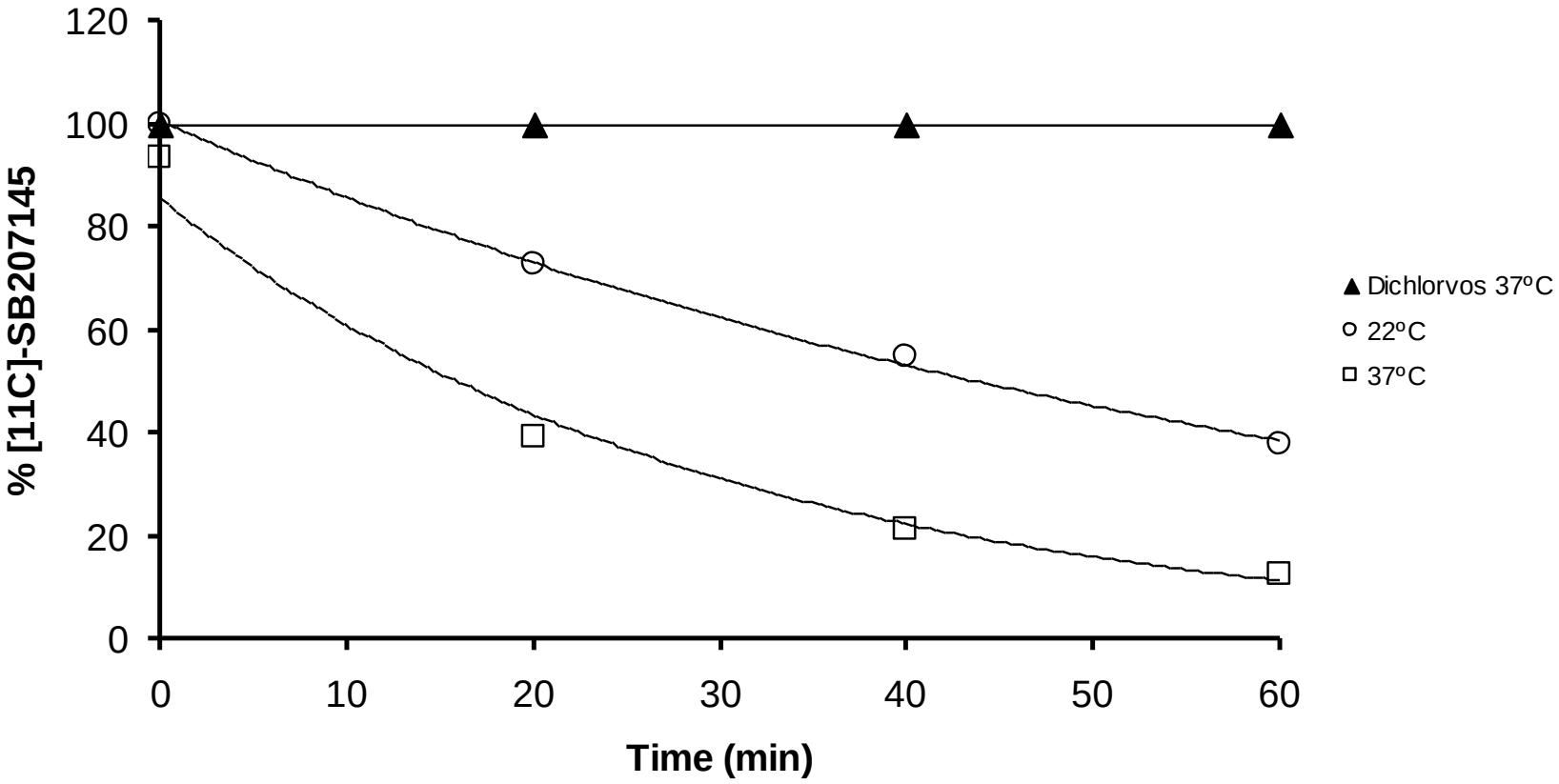
$$\text{tracer} = \frac{area(tracer)}{area(tracer) + area(metabolites)}$$

☹ The tracer ^{11}C-SB207145 is metabolized in plasma!

- Adding the esterase inhibitor dichlorvos to the blood samples stops the metabolism immediately

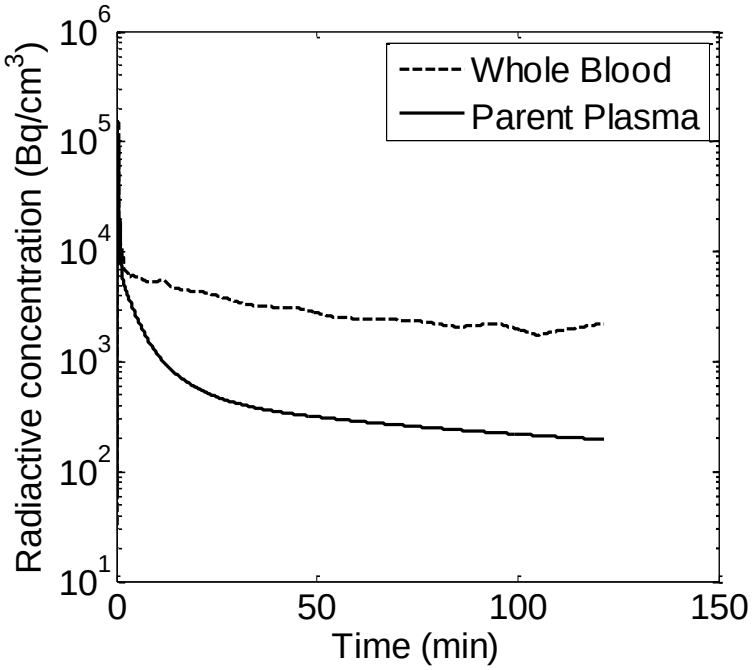
High **P**erformance **L**iquid **C**hromatography separate metabolites from parent compound

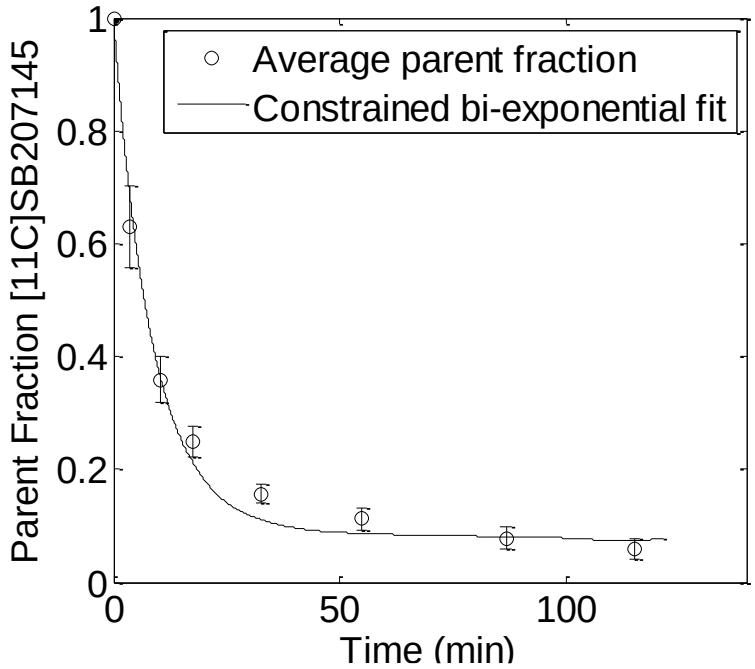
Compound stability in blood



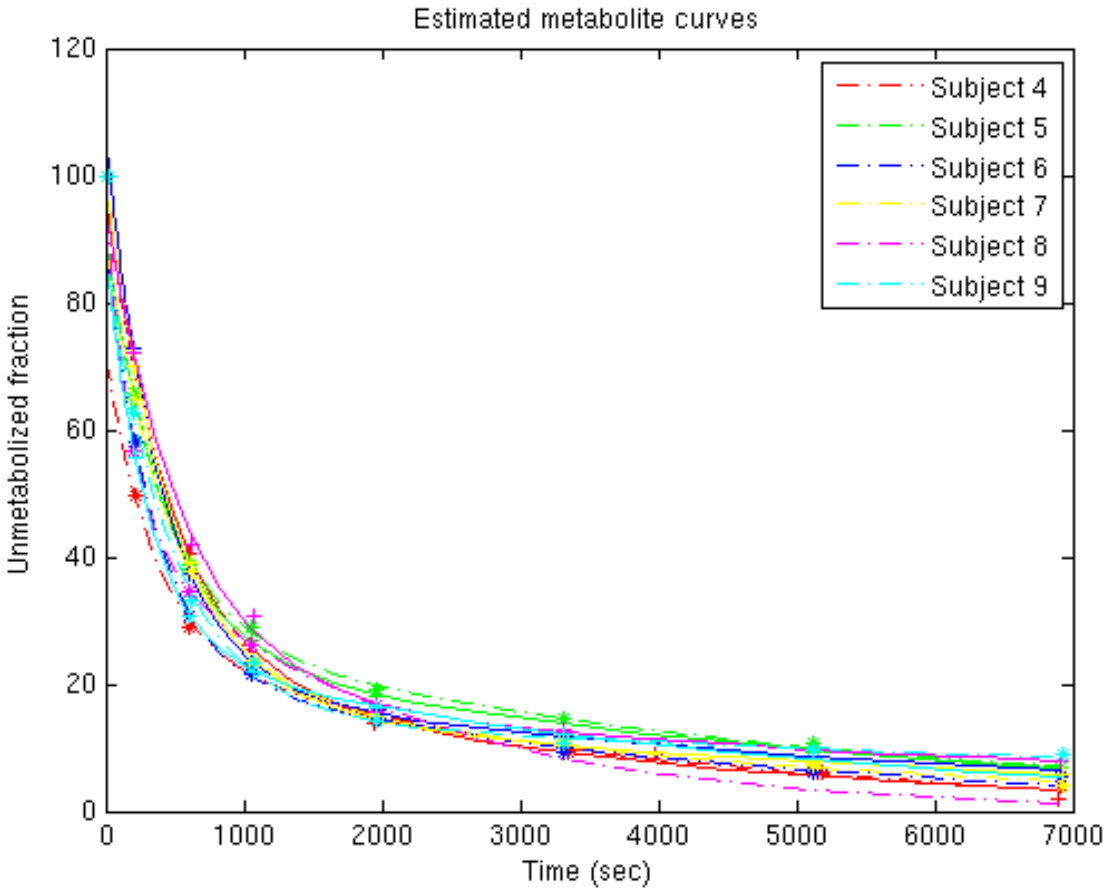
Marner et al, 2009 & 2010

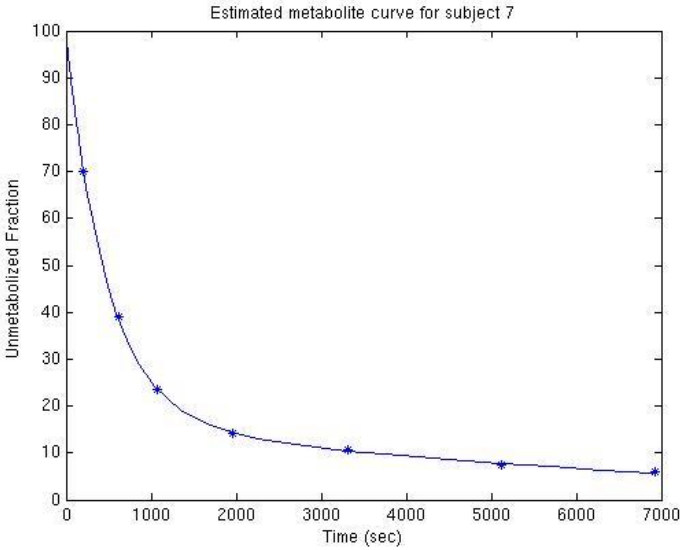
SB207145 systemic metabolism



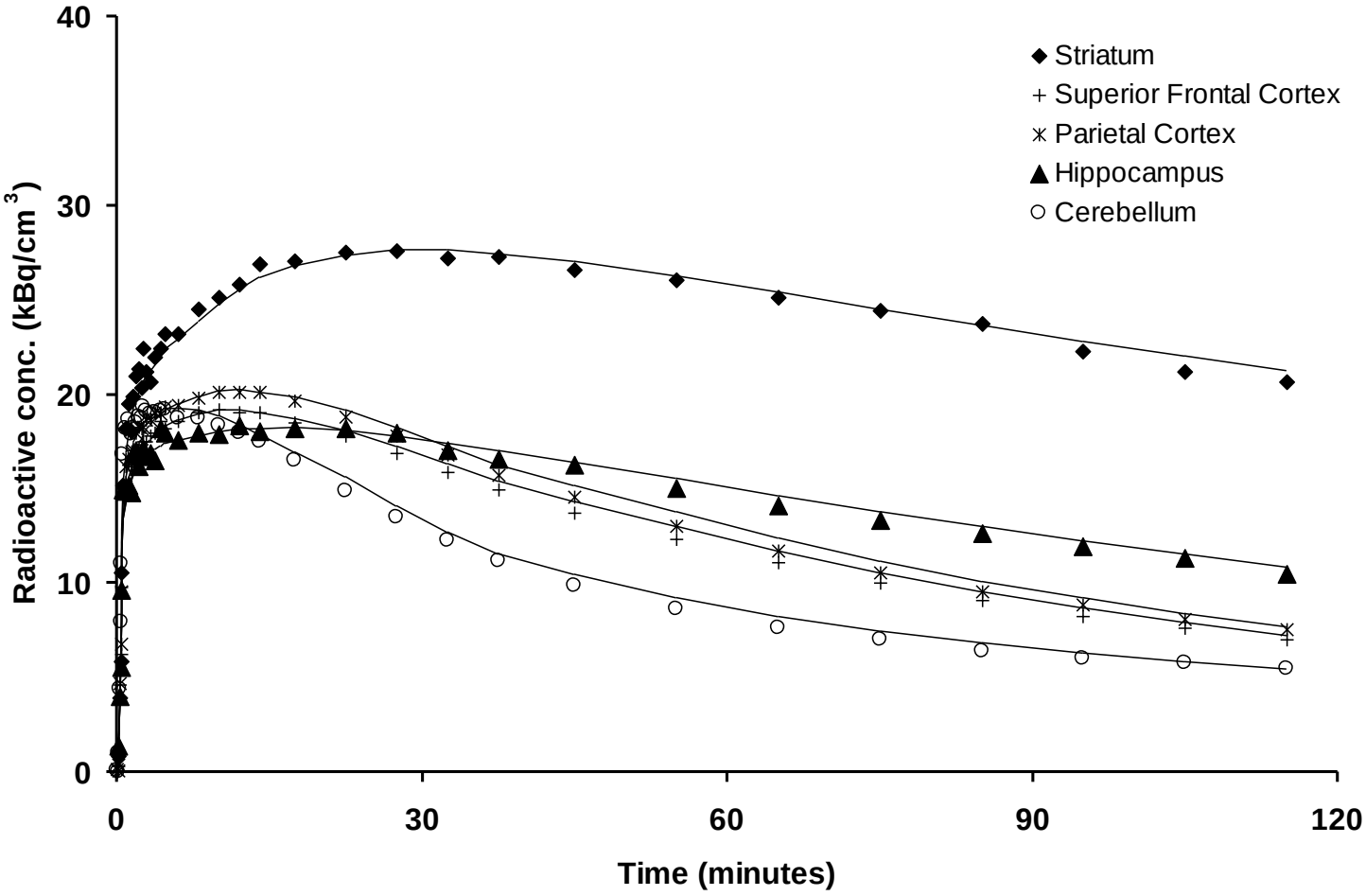


Metabolite Curves





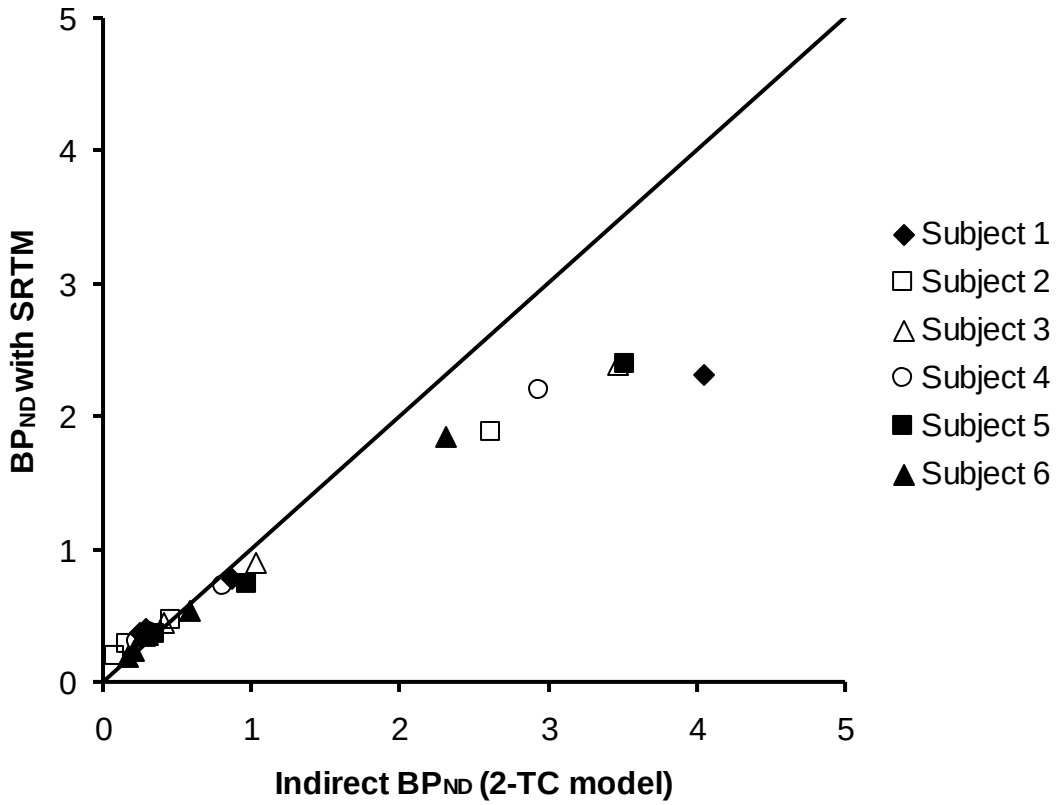
Time Activity Curves



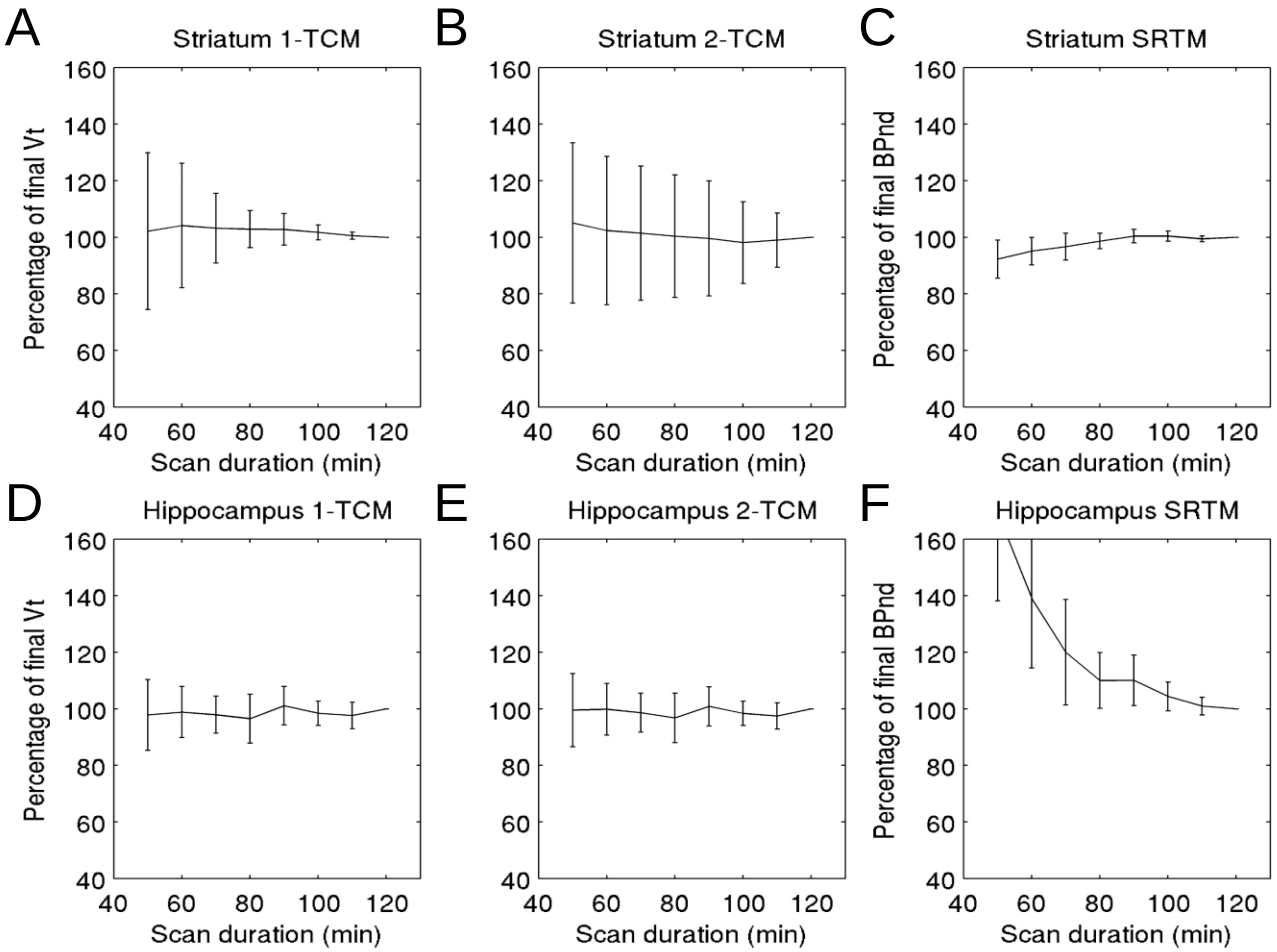
Results: Marked lower variability with the Simplified Reference Tissue Model

	2- Tissue Compartment Model		*Simplified Reference Tissue Model*	
Regions	Mean (SD)	Variability in test-retest (SD)	Mean (SD)	Variability in test-retest (SD)
Left caudatus	5.19 (1.03)	0.24 (0.20)	3.24 (0.35)	0.06 (0.04)
Right caudatus	5.22 (1.41)	0.32 (0.18)	3.25 (0.19)	0.04 (0.04)
Left putamen	4.25 (0.83)	0.22 (0.19)	3.05 (0.33)	0.08 (0.08)
Right putamen	4.51 (0.79)	0.14 (0.08)	3.14 (0.44)	0.05 (0.03)
Left hippocampus	0.91 (0.24)	0.19 (0.21)	0.78 (0.15)	0.05 (0.04)
Right hippocampus	0.88 (0.21)	0.29 (0.25)	0.77 (0.14)	0.17 (0.11)
Left parietal cortex	0.95 (0.26)	0.28 (0.19)	0.93 (0.09)	0.07 (0.07)
Right parietal cortex	0.95 (0.28)	0.26 (0.25)	0.92 (0.10)	0.07 (0.06)

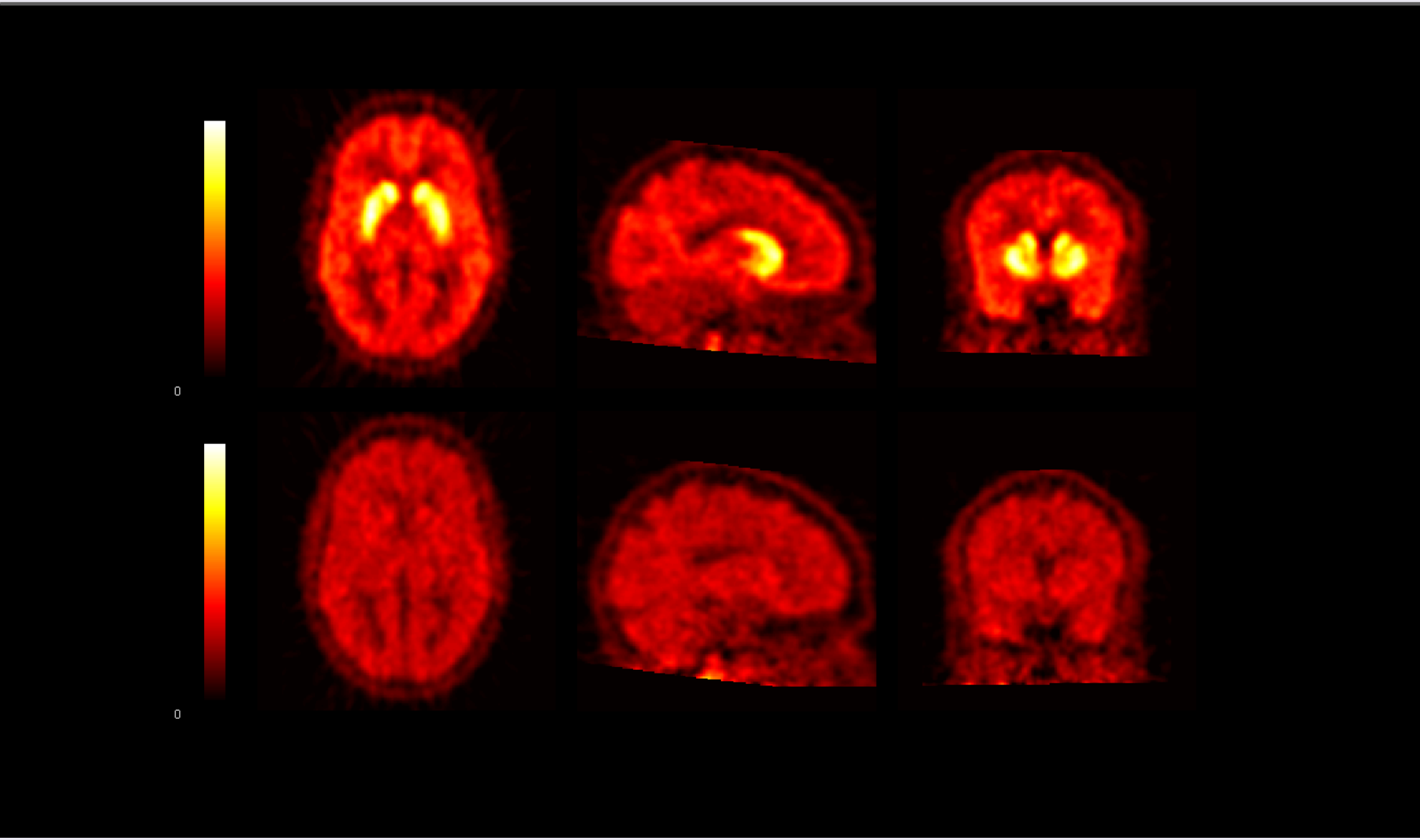
Results: Bias in high binding regions with the Simplified Reference Tissue Model



Scan length effects



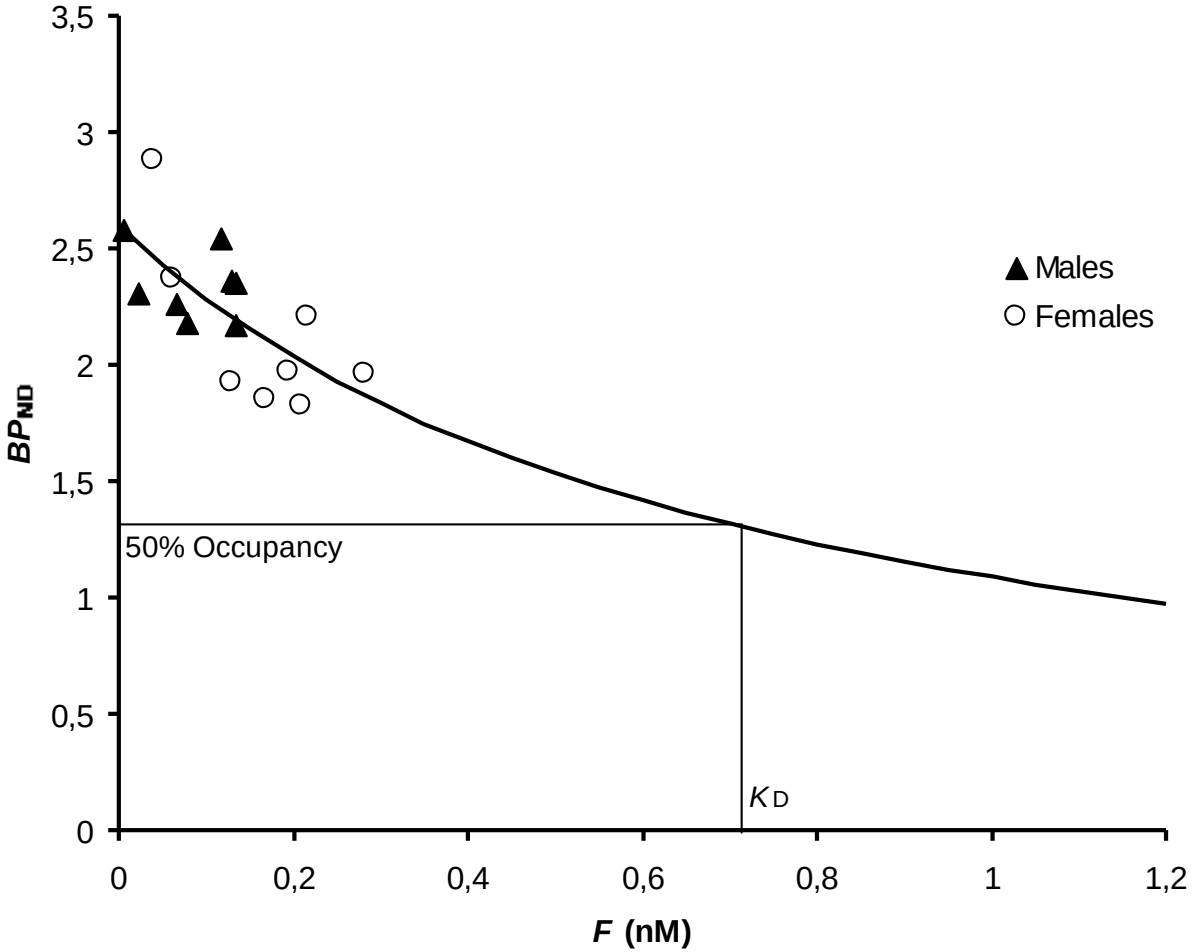
Blocking of 5-HT_4 receptors



Tracer Dose – How Do We Know?

- An in vivo saturation study to directly estimate the tracer dose limit: Escalating doses of ”cold” ligand in same individual or in a cohort
- Estimating tissue concentrations and relate to in vitro Kd or Bmax values

Occupancy – estimation of in vivo K_D

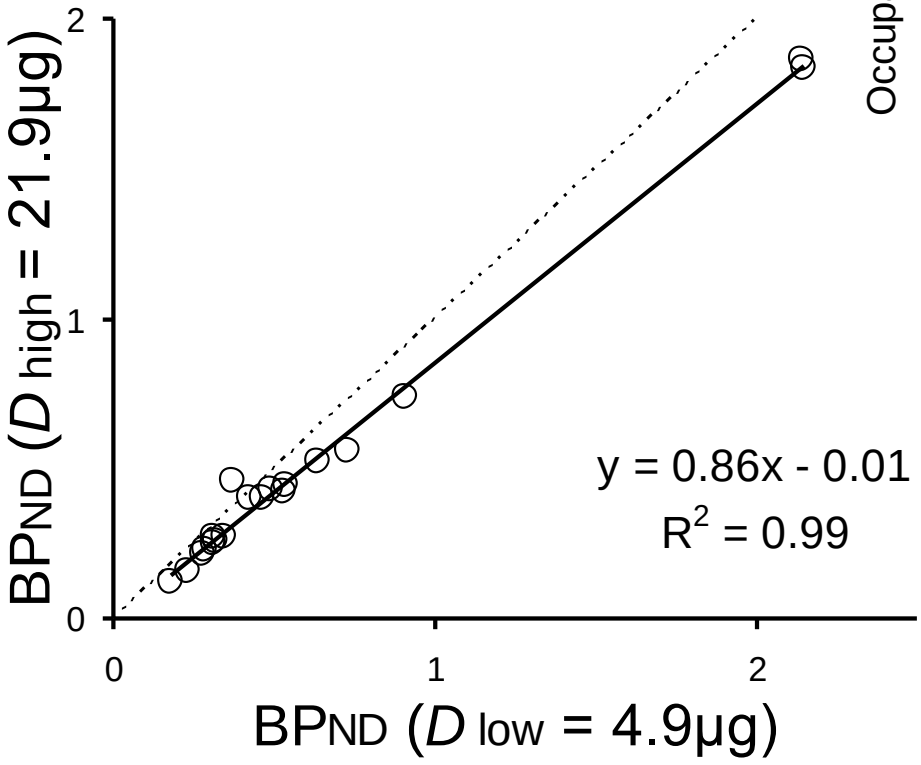


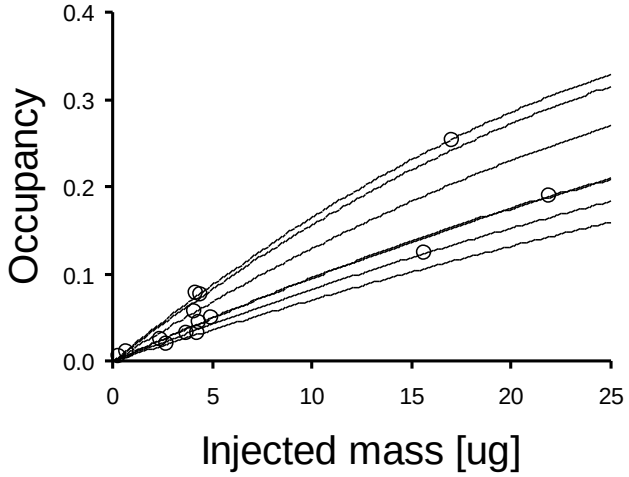
$$B = \frac{B_{\text{avail}} \cdot F}{F + K_D} \Leftrightarrow$$

$$\frac{B}{F} = \frac{B_{\text{avail}}}{F + K_D} \approx$$

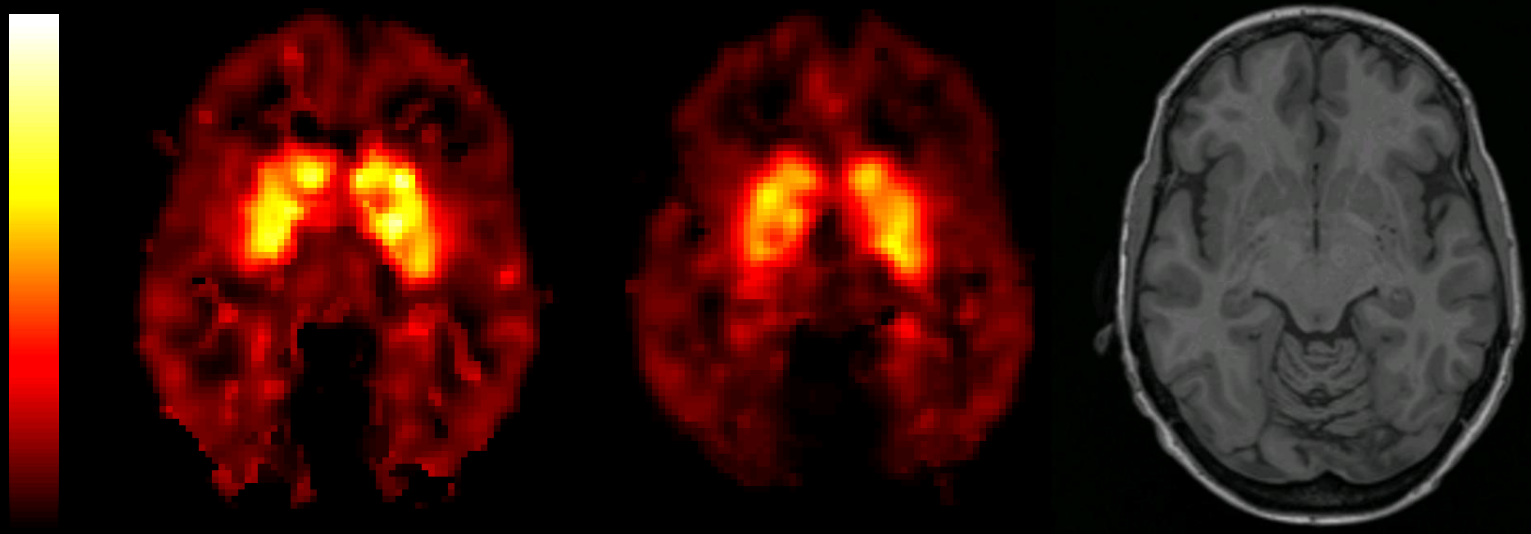
$$BP_{ND} = \frac{B_{\text{avail}}}{F + K_D}$$

Marner et al, 2009


BPND (D high = 21.9µg)
BPND (D low = 4.9µg)
y = 0.86x - 0.01
R2 = 0.99
0
1
2


Occupancy
Injected mass [ug]
0.0
0.1
0.2
0.3
0.4
0
5
10
15
20
25

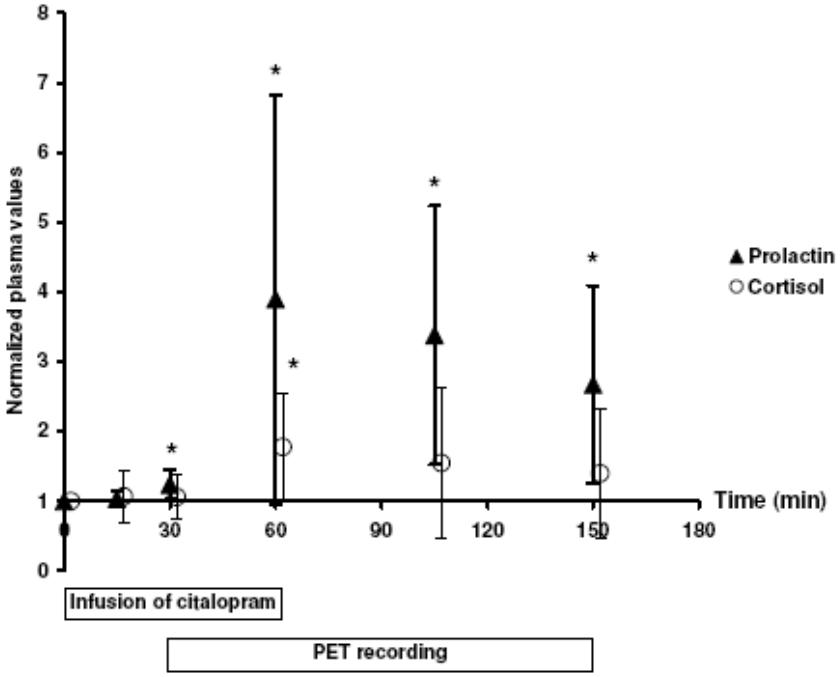
Mass doses

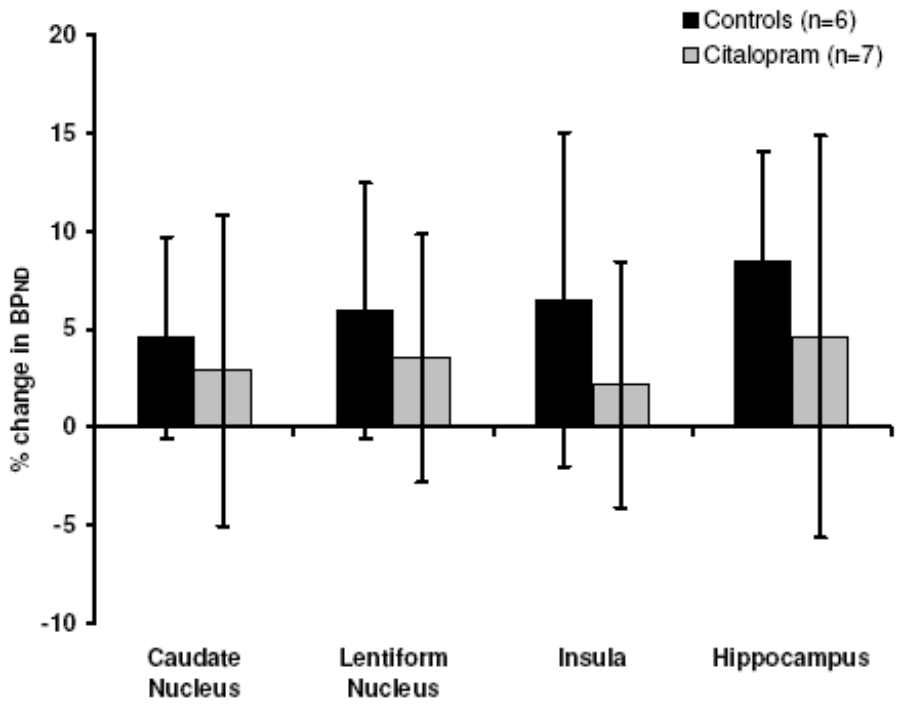


Parametric BP_{ND} images after injection of 4.9 µg ligand (left), 21.9 µg ligand (middle) and the corresponding MRI (right).
BP_{ND} decreased by 14% as a result of mass dose.

	Mass Dose [µg]		Occupancy plots		Mass dose limits [µg]		
	D_{Low}	D_{High}	α	R^2	ID_{50}	D_5	D_{10}
Subject 1	4.9	21.9	0.85	0.99	94.3	5.0	10.5
Subject 2	4.2	17.0	0.80	0.97	50.2	2.6	5.6
Subject 3	3.7	15.6	0.91	0.99	110.7	5.8	12.3
Subject 4	0.3	4.4	0.93	0.99	53.6	2.8	6.0
Subject 5	0.7	4.1	0.95	1.00	66.9	3.5	7.4
Subject 6	2.4	4.3	0.98	0.99	93.7	4.9	10.4
Subject 7	2.7	4.2	0.99	0.99	131.1	6.9	14.6
Mean ± SD	2.7 ± 1.7	10.2 ± 7.7	0.92 ± 0.07		85.8 ± 30.2	4.5 ± 1.6	9.5 ± 3.3

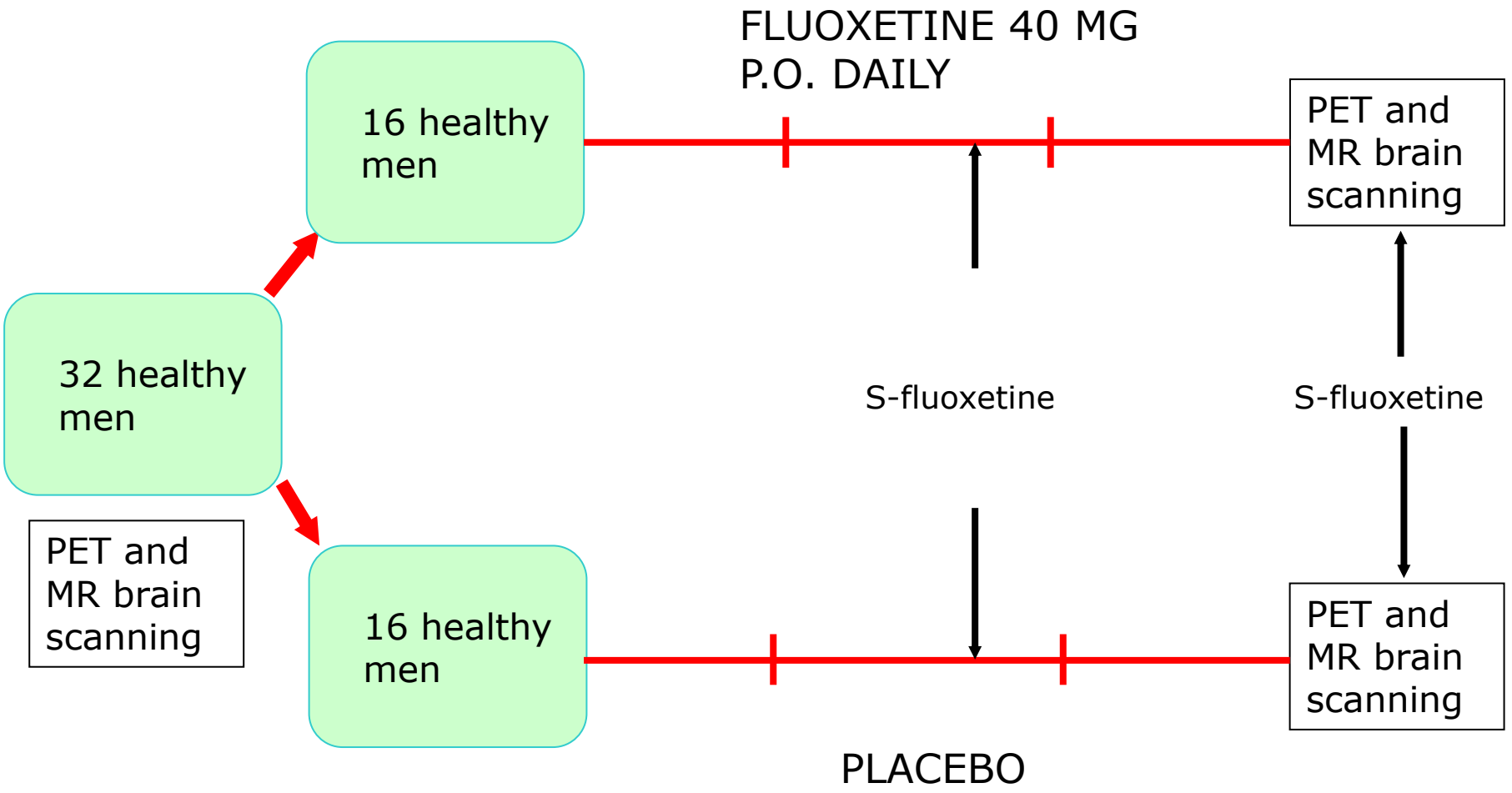
5-HT challenge with Citalopram infusion





Marner et al, 2010

SSRI 3-Weeks Intervention Study



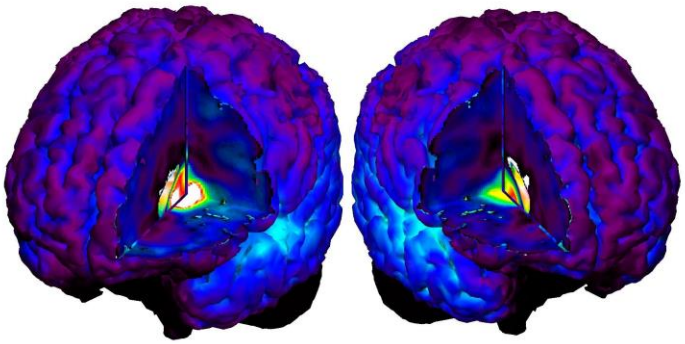
Haahr et al, Mol Psych 2014

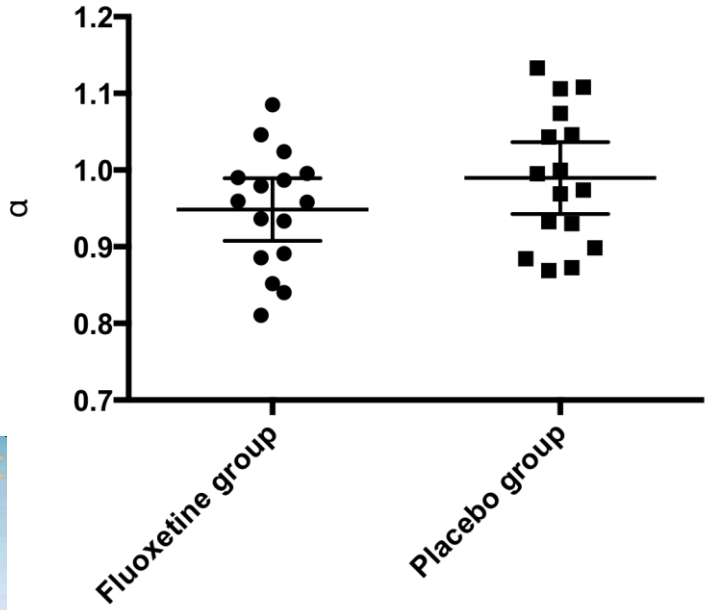
SSRI project: Hypotheses

- Evaluate effects of 3-week SSRI administration on underlying brain function and brain chemistry
  - **Hypothesis 1**: SSRI administration will result in a significant decrease in 5-HT4R binding in the SSRI intervention group, but not in the placebo group (*Pharmacological challenge effect*)

The 5-HT$_4$ receptor reflects brain 5-HT levels

The 5-HT4 receptor binding decreases after 3 weeks of fluoxetine intervention





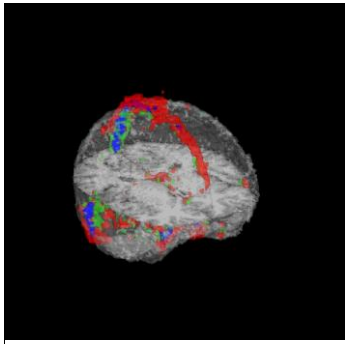
Haahr et al, Mol Psych 2014

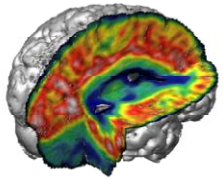
Conclusions

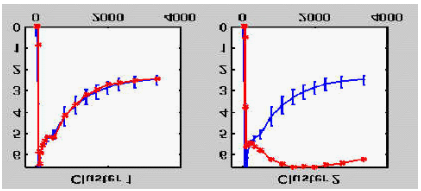
- ^{11}C-SB207145 has a high target-to-background signal – also in low binding regions
- The regional distribution of ^{11}C-SB207145 corresponds to 5-HT$_4$ receptor density
- The tracer is sufficiently reversible to allow for quantification, also in high binding regions – but long scan length (120 min) is required
- The Simplified Reference Tissue Model (without arterial cannulation and metabolite measurements) can be used for estimation of binding potentials. Test-retest variability is between 6 and 10%. However, a bias of 27-36% must be expected in high binding regions
- Cerebellum is valid as a reference region (complete blocking)
- Injected mass of SB207145 should be kept <5ug to ensure <5% receptor occupancy
- The tracer is susceptible to chronic, but not acute changes in the endogenous neurotransmitter

Quantification Methods

- Bolus injection with kinetic modelling
  - with arterial input function
  - with vascular voxel input function
  - with reference tissue input function
  - with standardized input function
  - other (venous, heart, carotid)
- Bolus-infusion method
  - with venous blood samples
  - with reference tissue



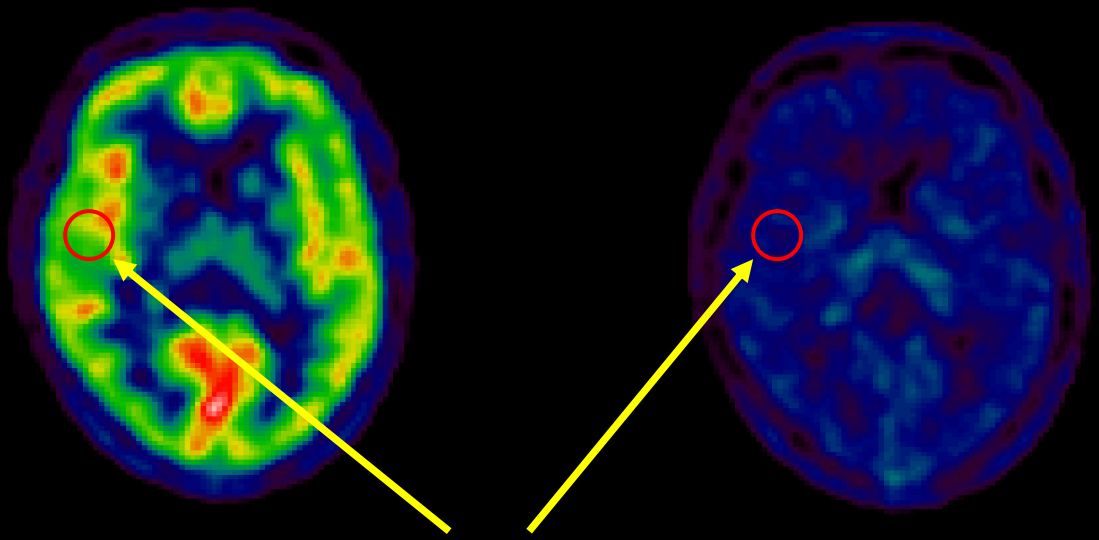




Ways to determine V_{ND}

- labeled non-specific stereoisomer of the ligand
- tissue reference region
- complete receptor blockade
- brain:blood partition coefficient, ρ
- from the V_T occupancy plot

5-HT_{2A} Receptor Blockade with Ketanserin



$$\text{Occupancy} = (BP_{P\ (unblocked)} - BP_{P\ (blocked)})/BP_{P\ (unblocked)}$$

$$= 1 - (BP_{P\ (blocked)}/BP_{P\ (unblocked)})$$

^{18}F-altanserin PET, Pinborg et al (2004)

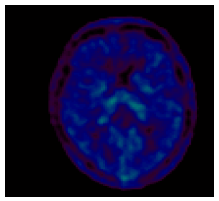
Occupancy

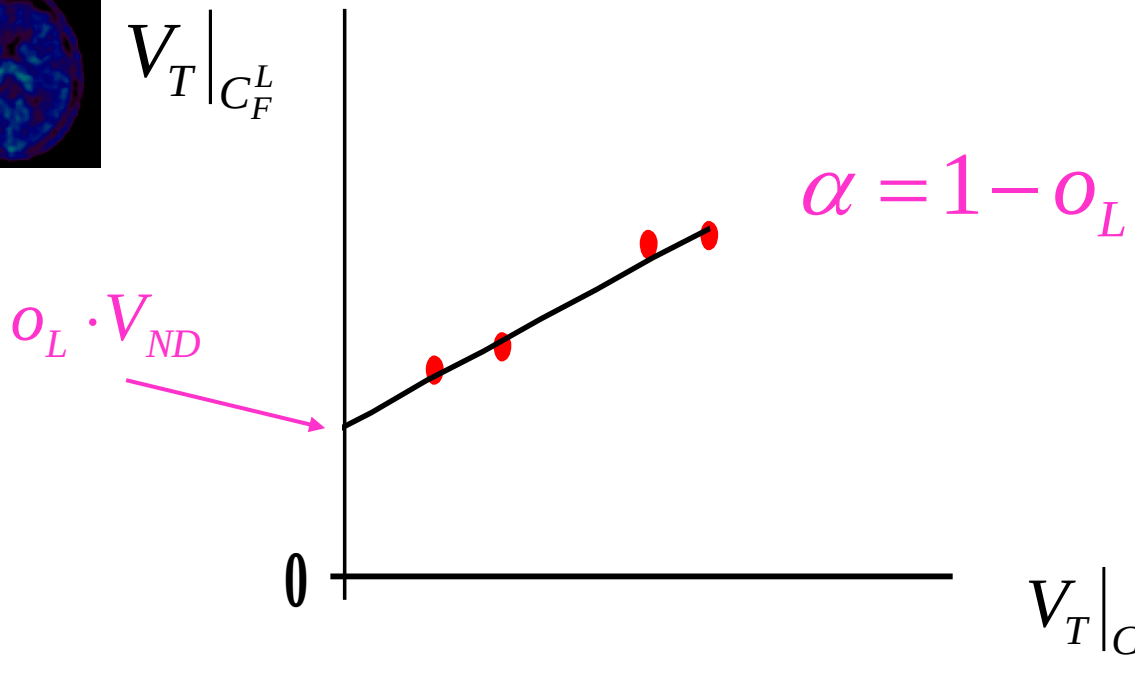
Unblocked $$V_T\Big|_{C_F^L=0} = V_S + V_{ND}$$

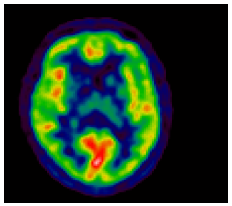
Blocked $$V_T\Big|_{C_F^L} = V_S(1-O) + V_{ND}$$

Lassen JCBFM 1992

V_T Occupancy Plot

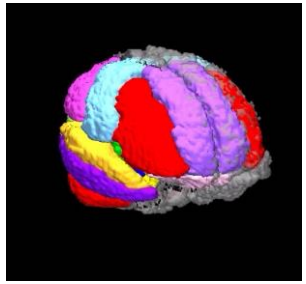






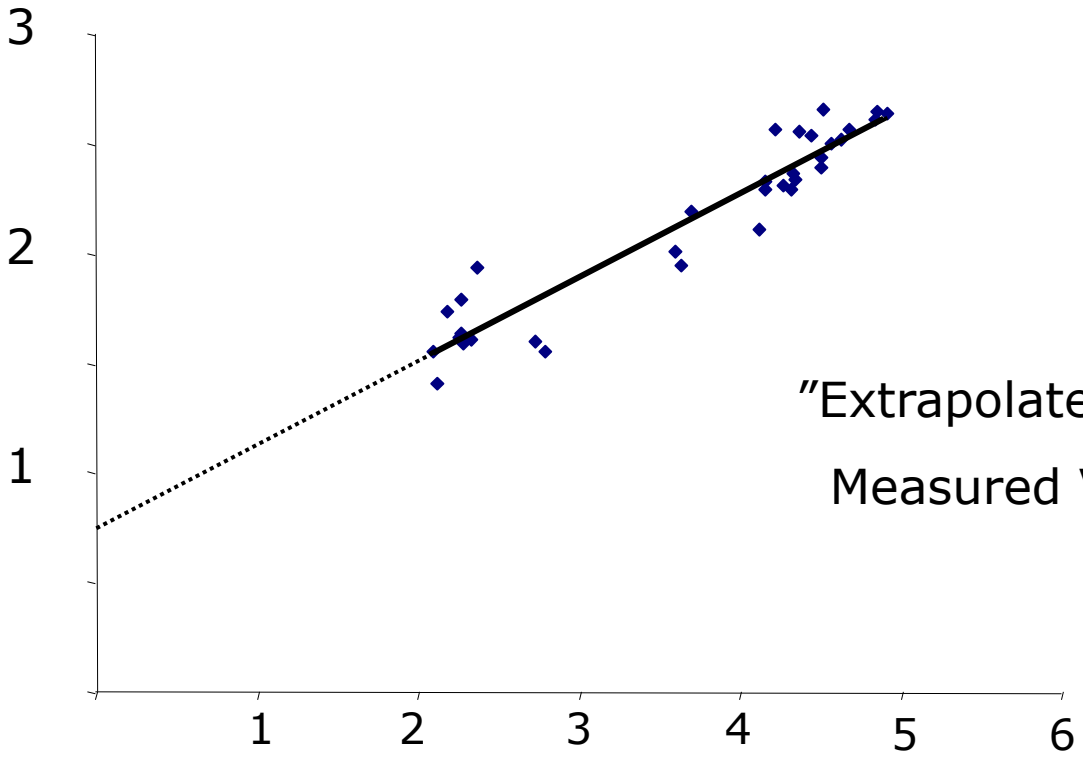
$$V_T\big|_{C_F^L} = o_L \cdot V_{ND} + (1 - o_L) V_T\big|_{C_F^L=0} \qquad \text{Eq. 9.15}$$

5-HT_{2A} Receptor Quetiapine Occupancy



$y = 0.732 + 0.383x$

$r^2 = 0.907$



"Extrapolated" V_{ND} = 1.2

Measured V_{ND} = 1.9

Rasmussen et al, Psychopharm, 2010