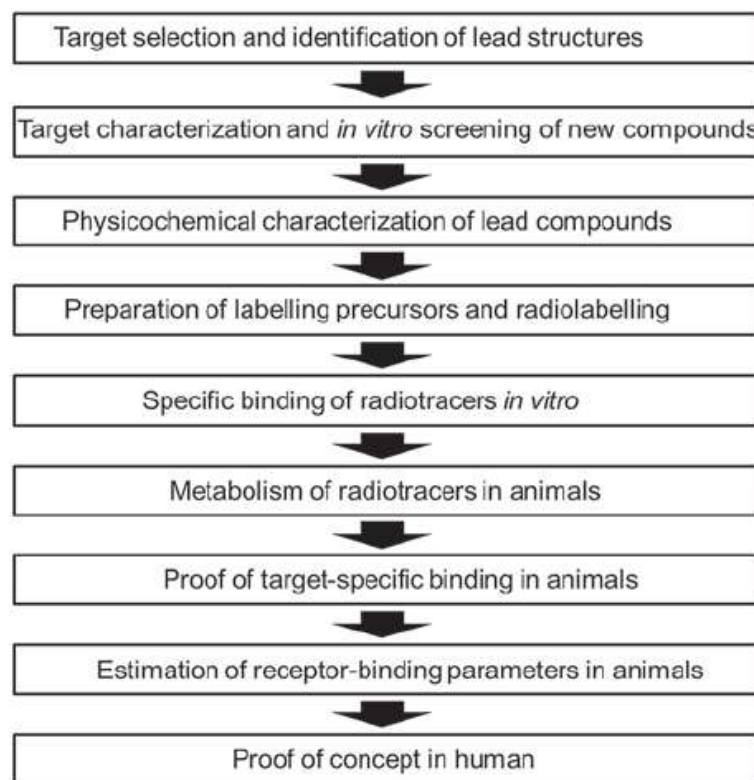


How to establish a new radiotracer

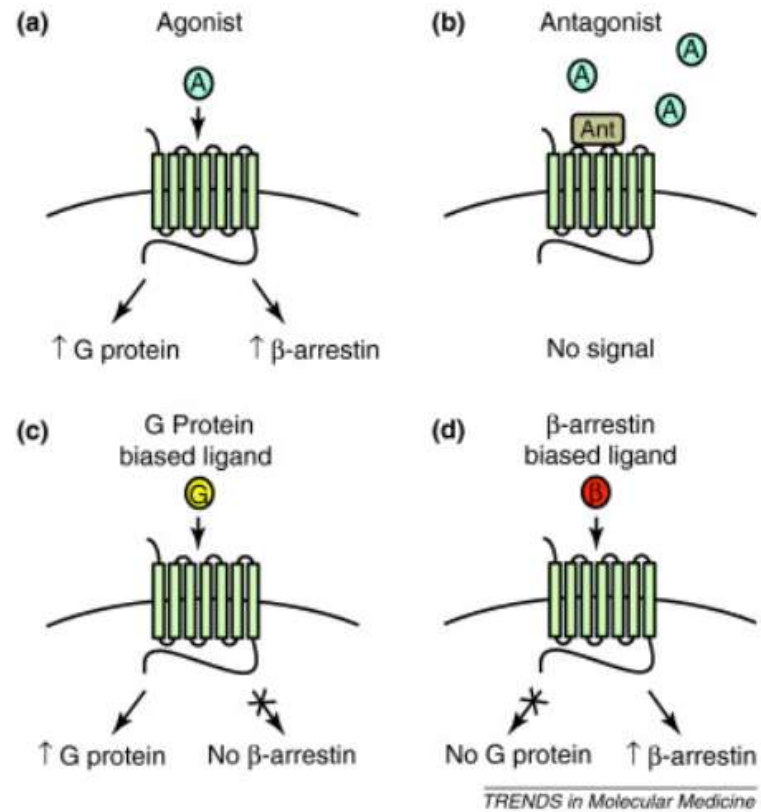


Brust et al, 2014

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**
 - 1TC, 2TC, linear methods, reference tissue methods...
- **Mass dose**

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



Whalen EJ et al, TIMolMed 2011

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



Journal of Cerebral Blood Flow & Metabolism (2010) 30, 1682–1706
© 2010 ISCBFM All rights reserved 0271-678X/10 \$32.00
www.jcbfm.com

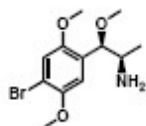
Review Article

Measuring endogenous 5-HT release by emission tomography: promises and pitfalls

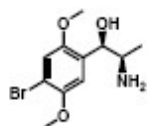
Louise M Paterson¹, Robin J Tyacke¹, David J Nutt¹ and Gitte M Knudsen²

¹Division of Experimental Medicine, Neuropsychopharmacology Unit, Imperial College London, Burlington Danes Building, London, UK; ²Neurobiology Research Unit and Center for Integrated Molecular Brain Imaging, Rigshospitalet and University of Copenhagen, Copenhagen, Denmark

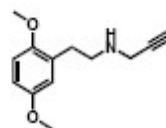
5-HT_{2A} Receptor Compounds



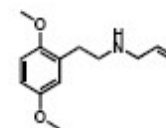
Cimbi 1



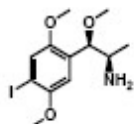
Cimbi 2



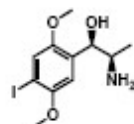
Cimbi 9



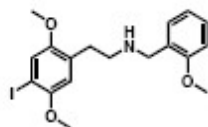
Cimbi 10



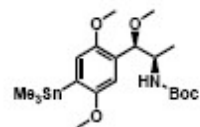
Cimbi 3



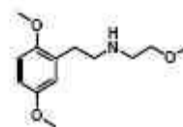
Cimbi 4



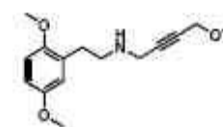
Cimbi 5



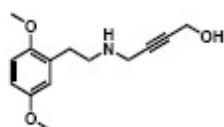
Cimbi 6



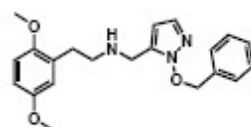
Cimbi 11



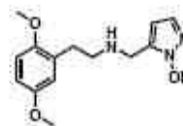
Cimbi 12



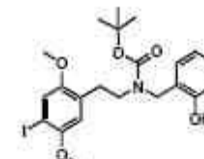
Cimbi 7



Cimbi 8



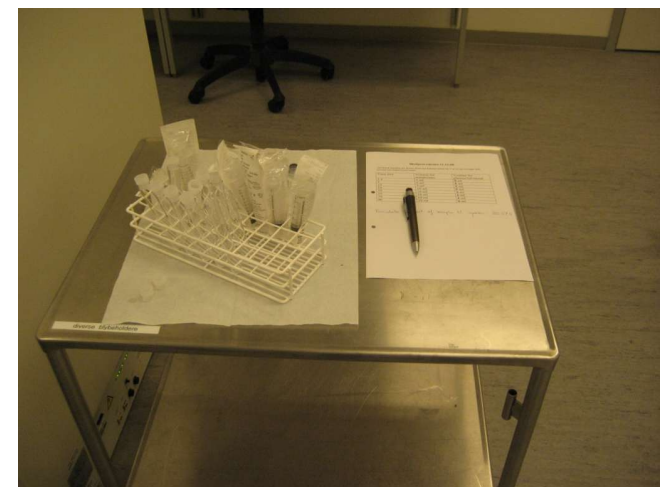
Cimbi 13



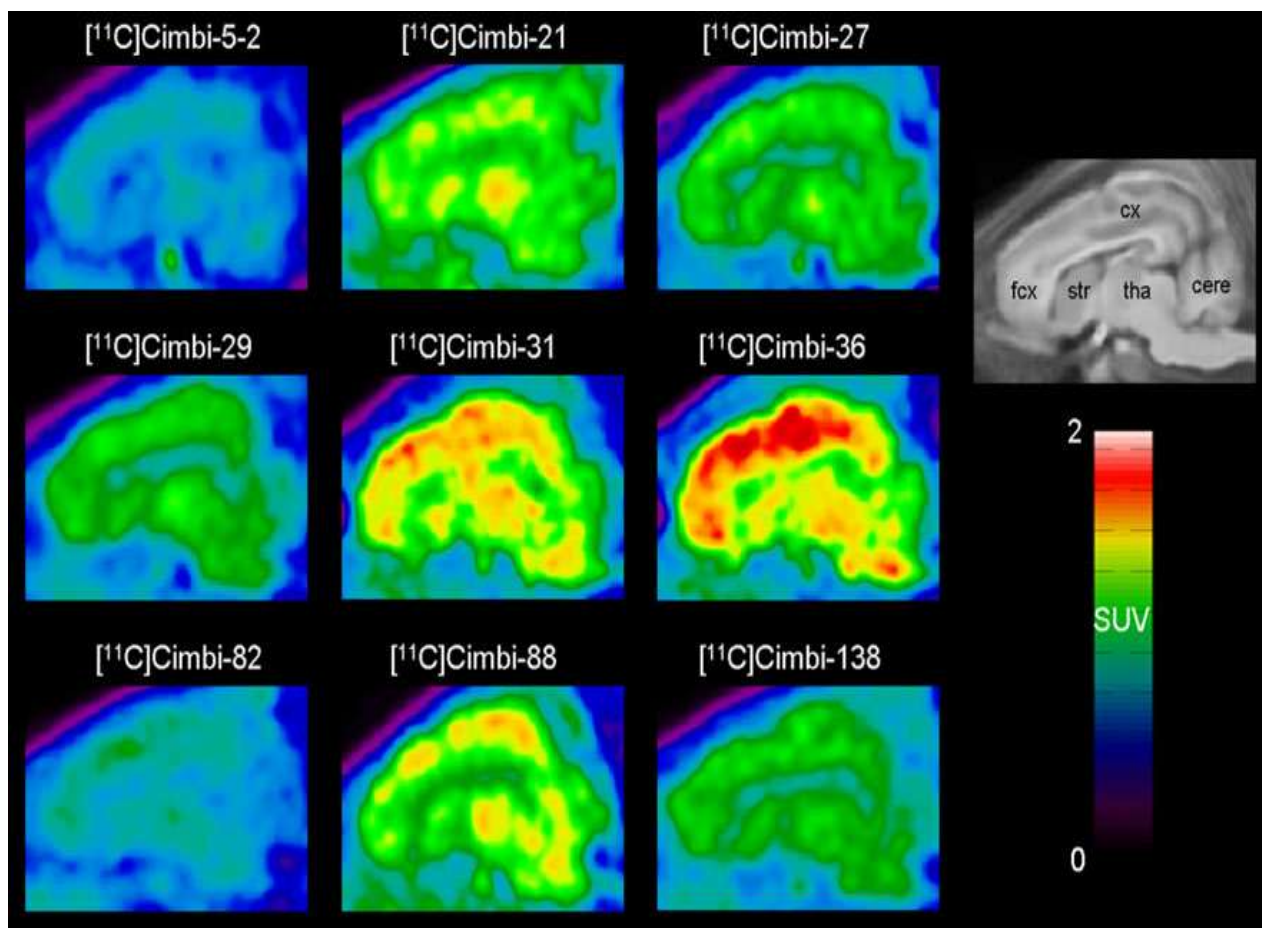
Cimbi 14

PET methods: Pigs

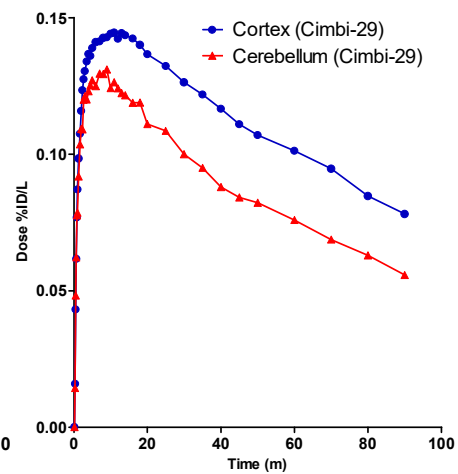
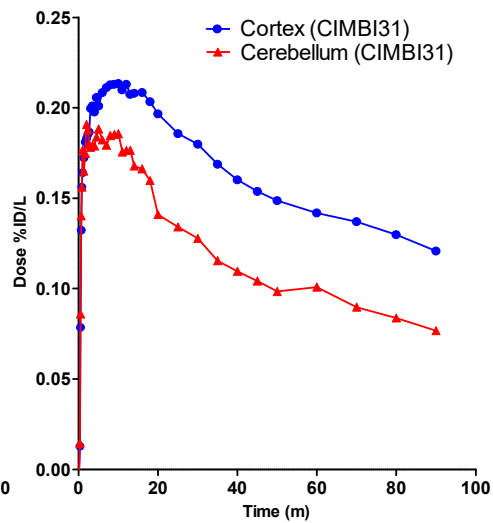
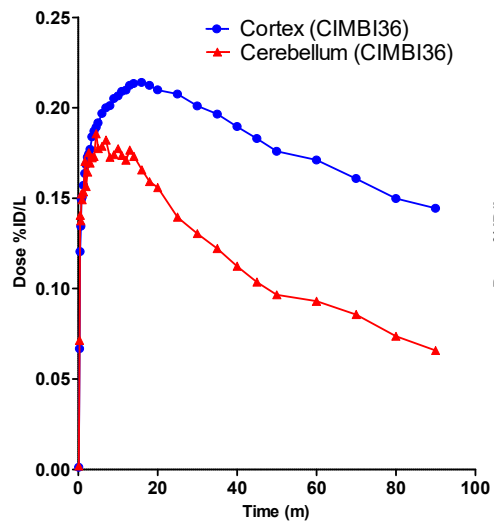
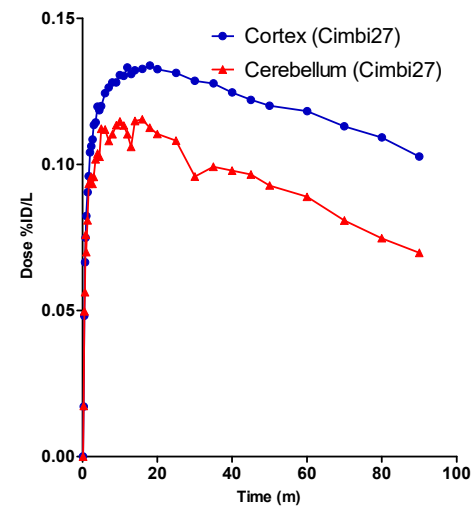
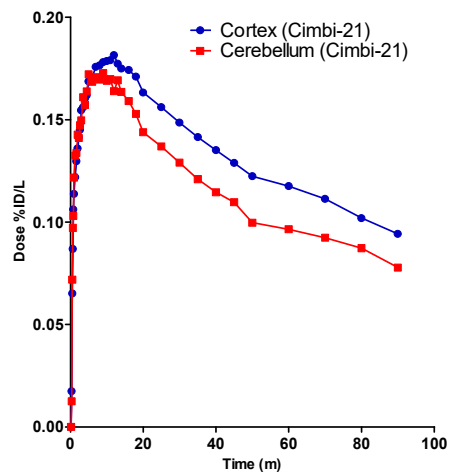
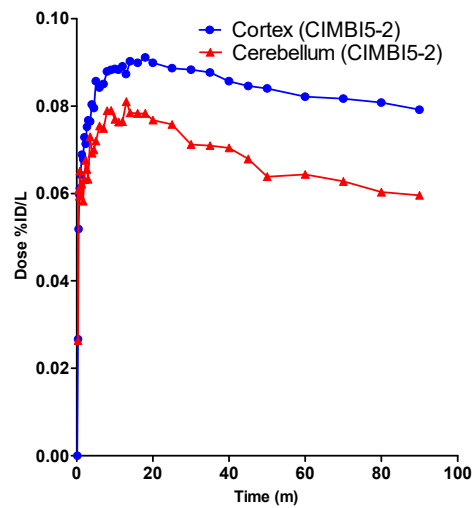
- Seven Danish Landrace Pigs (Weight 17.3 ± 0.76 kg)
- Anaesthesia: i.v. propofol
- Venous access through the milk vein, [^{11}C]Cimbi-5 given by IV bolus injections
- Monitoring blood pressure, heart rate, and temperature during the scans
- Catheter in the femoral artery: Input using an autosampler, blood and plasma sampling manually



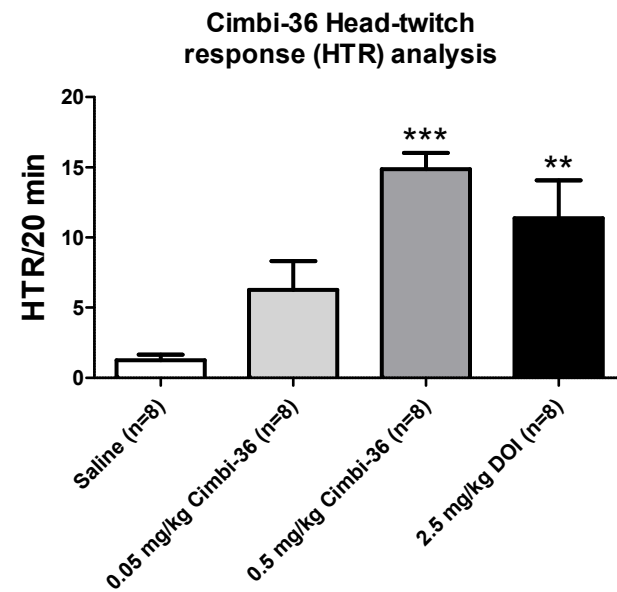
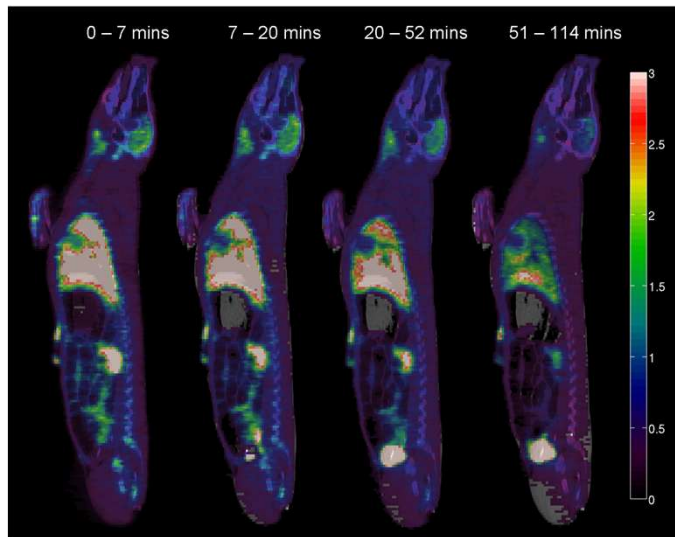
Nine ^{11}C -labelled 5-HT_{2A} receptor agonists



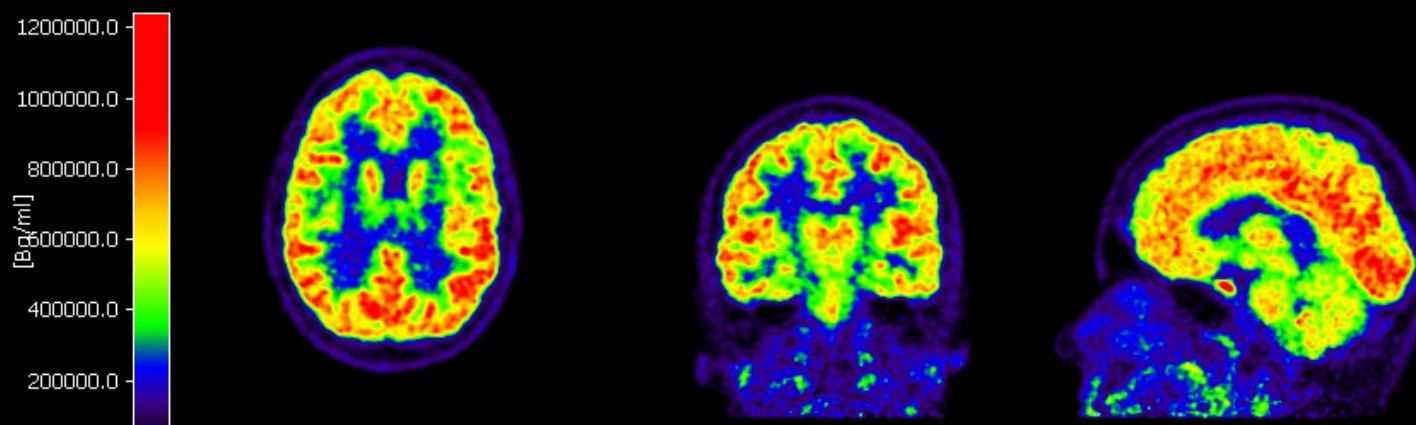
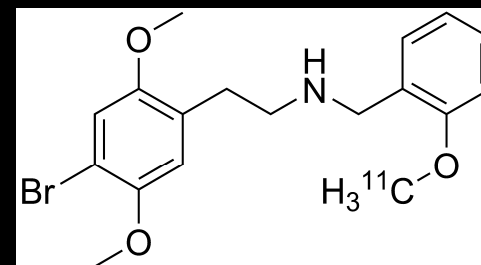
Ettrup et al, Eur J Nucl Med Mol Imaging, 2011



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

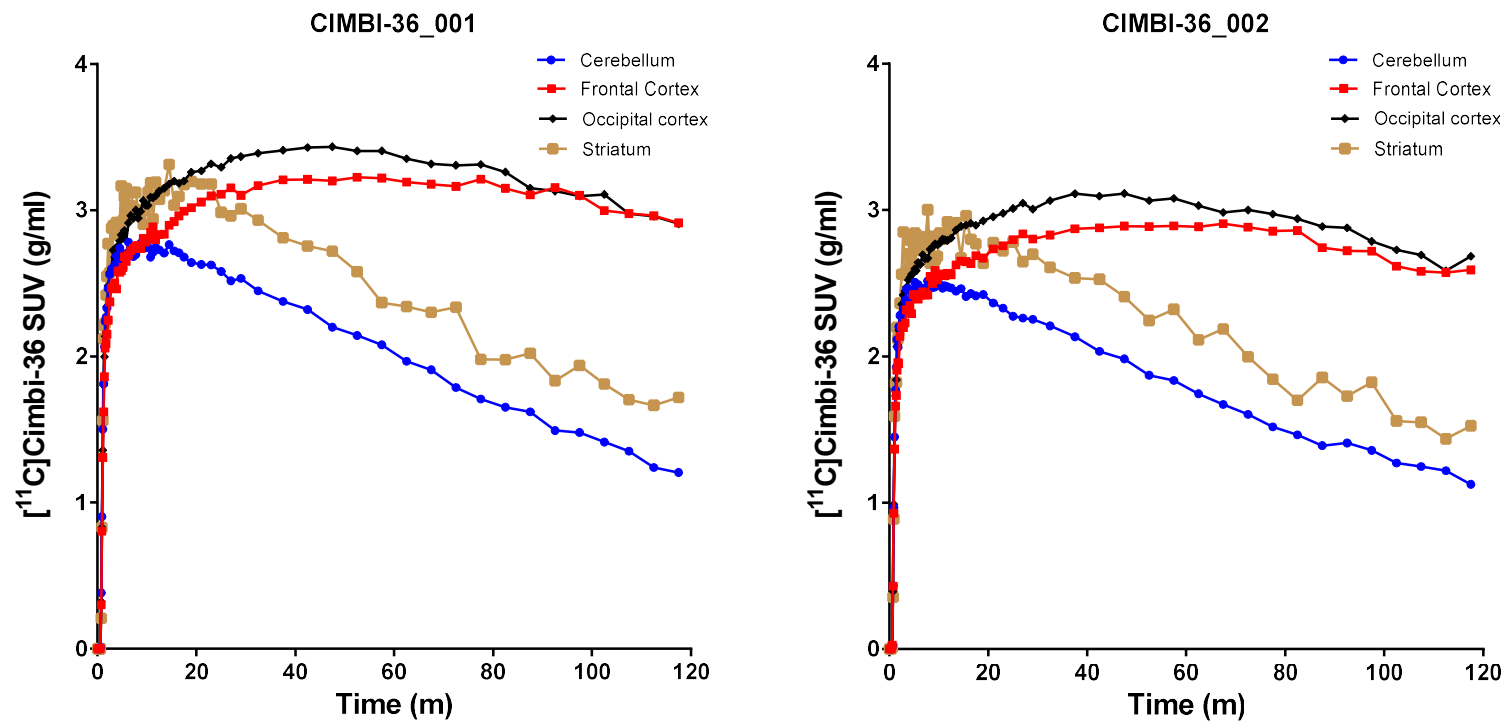


Ettrup et al, Mol Imaging Biol, 2013



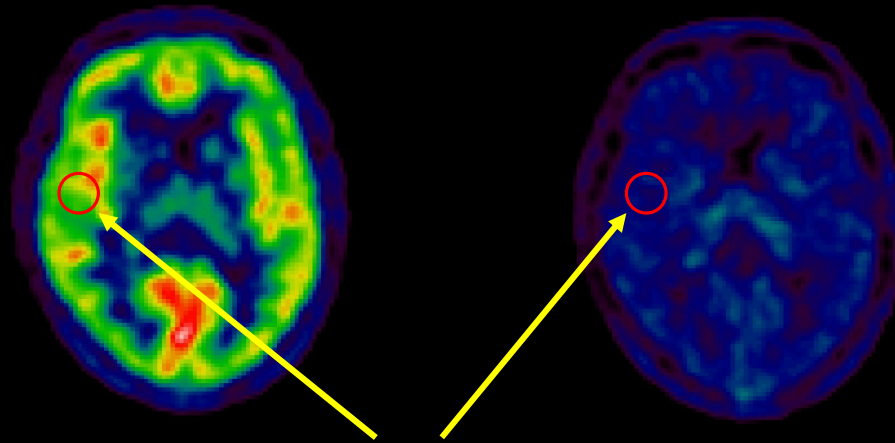
PET- and Cyclotron Unit, RH, 2012

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).

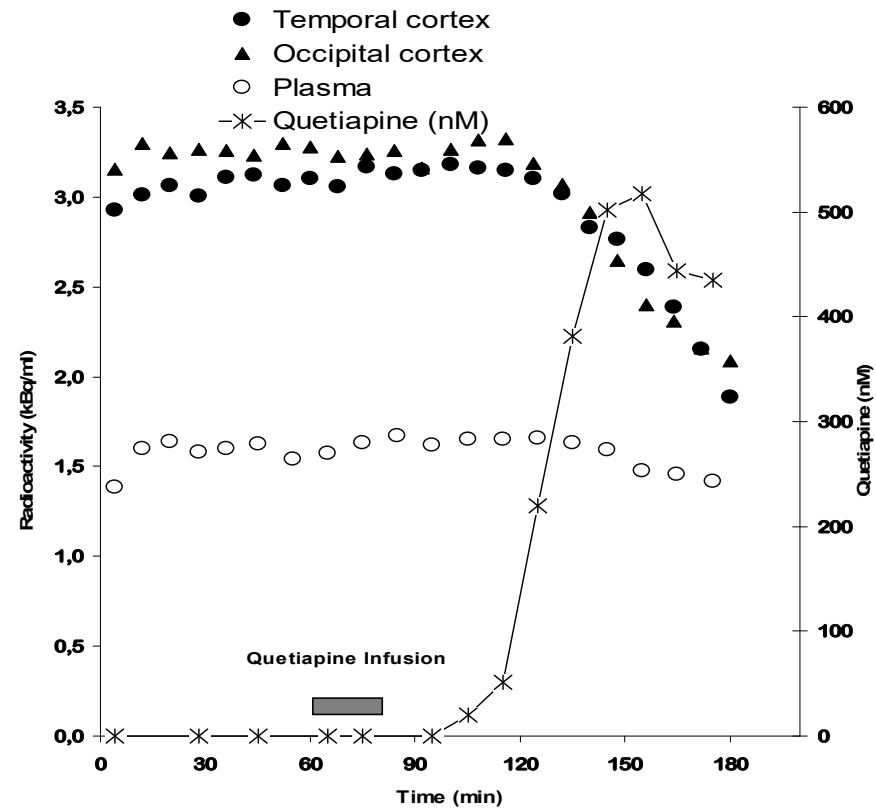
5-HT_{2A} receptor blockade with ketanserin



$$\begin{aligned}\text{Occupancy} &= (BP_{P(\text{unblocked})} - BP_{P(\text{blocked})}) / BP_{P(\text{unblocked})} \\ &= 1 - (BP_{P(\text{blocked})} / BP_{P(\text{unblocked})})\end{aligned}$$

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

Dynamic Occupancy Study



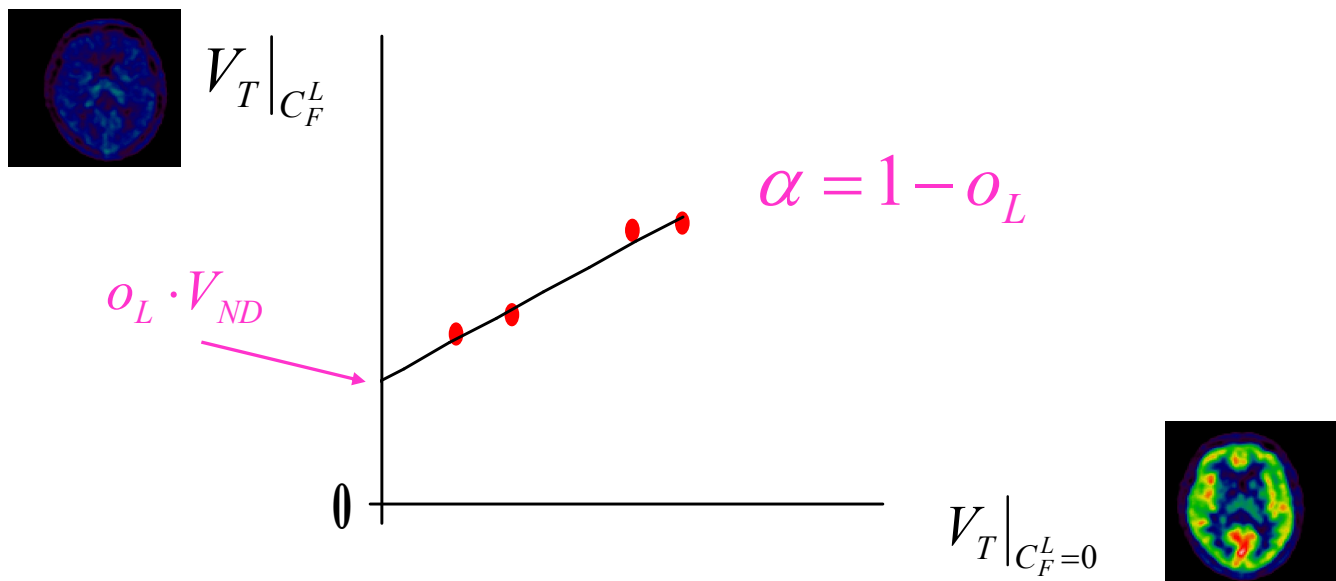
Rasmussen et al, Psychopharmacology 2011

Occupancy

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

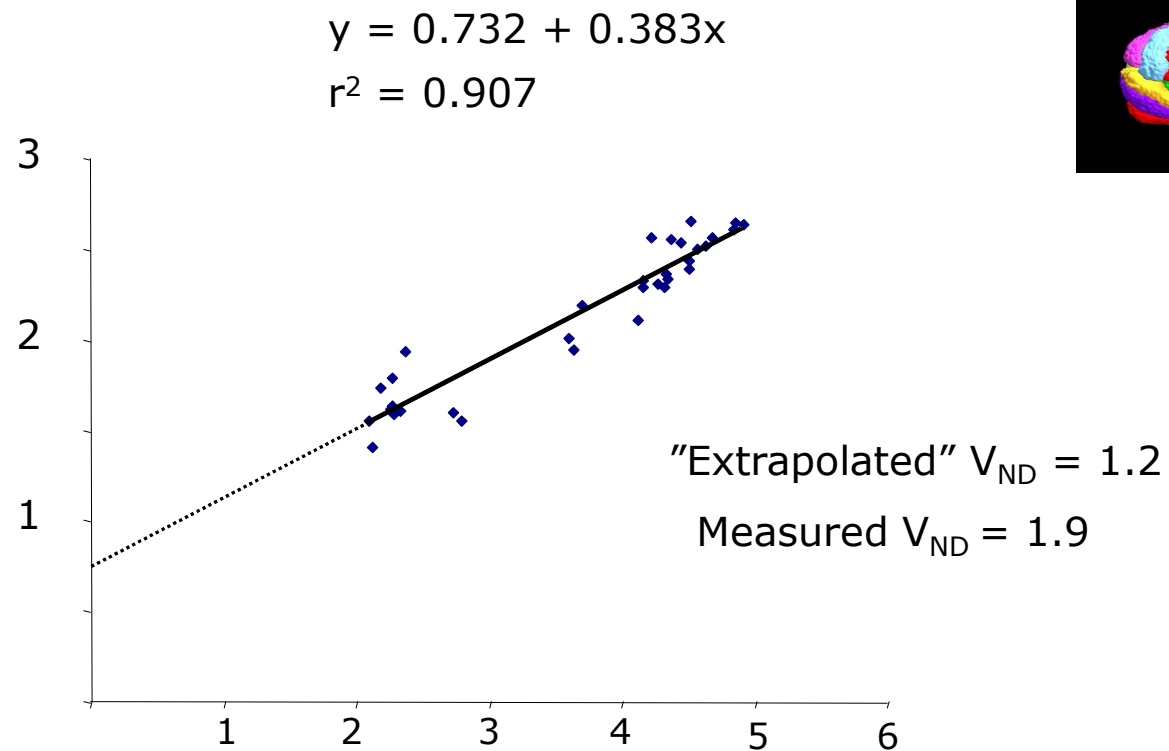
Blocked $V_T \big|_{C_F^L} = V_S (1 - O) + V_{ND}$

V_T Occupancy Plot



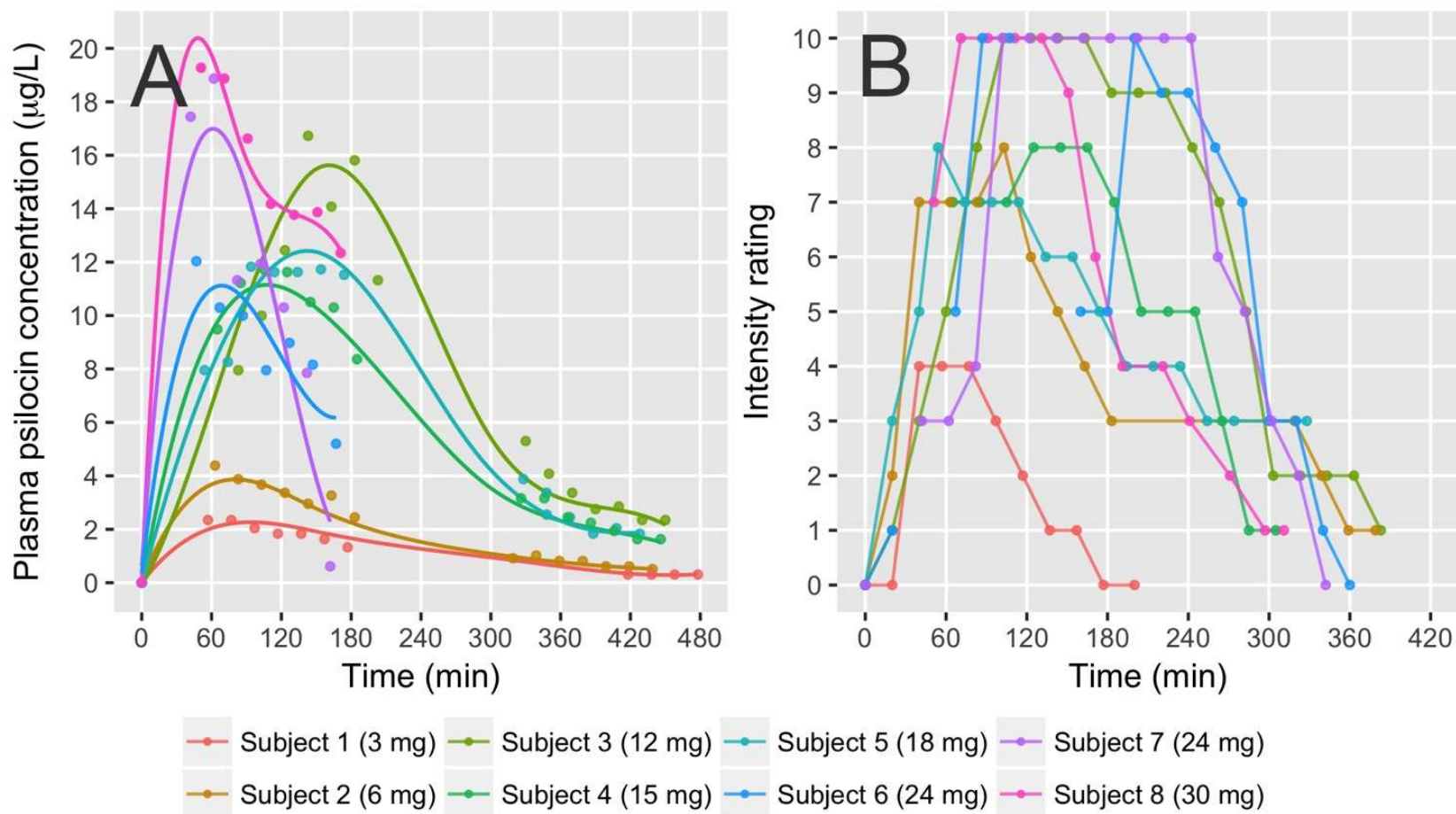
$$V_T |_{C_F^L} = o_L \cdot V_{ND} + (1 - o_L) V_T |_{C_F^L=0}$$

5-HT_{2A} Receptor Quetiapine Occupancy



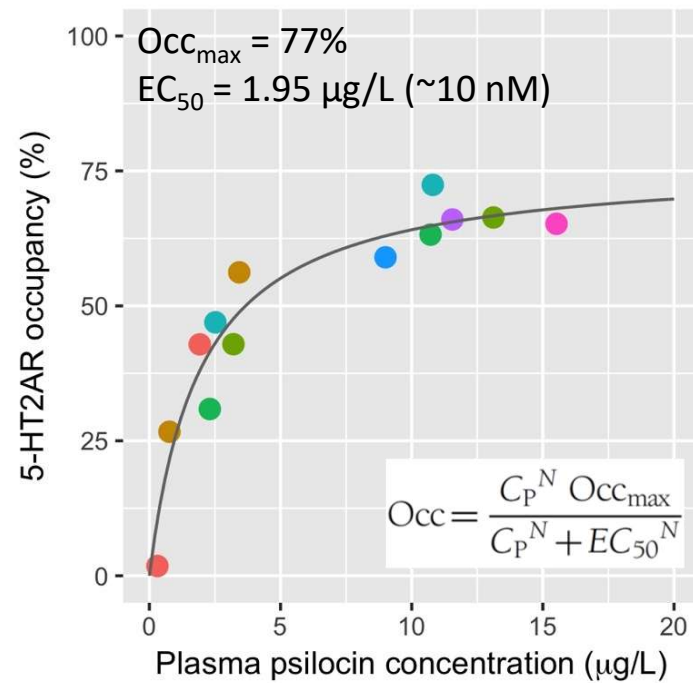
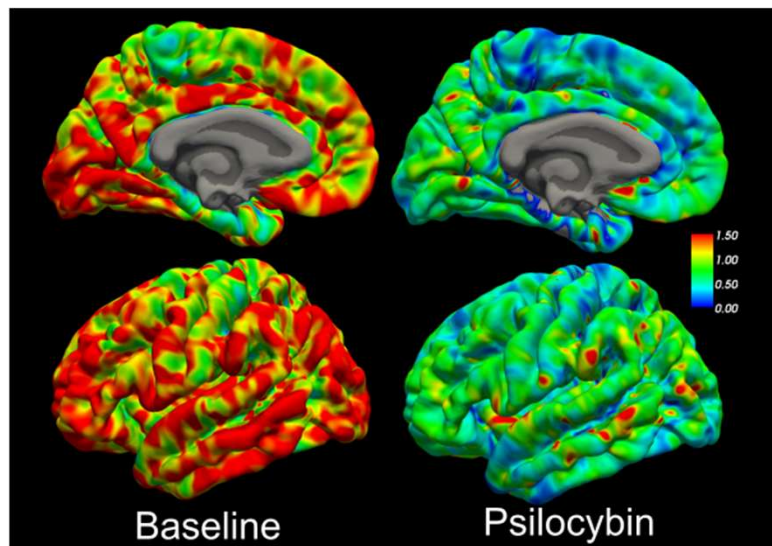
Rasmussen et al, Psychopharm, 2010

5-HT_{2A}R agonist psilocybin: Drug levels and subjective effects



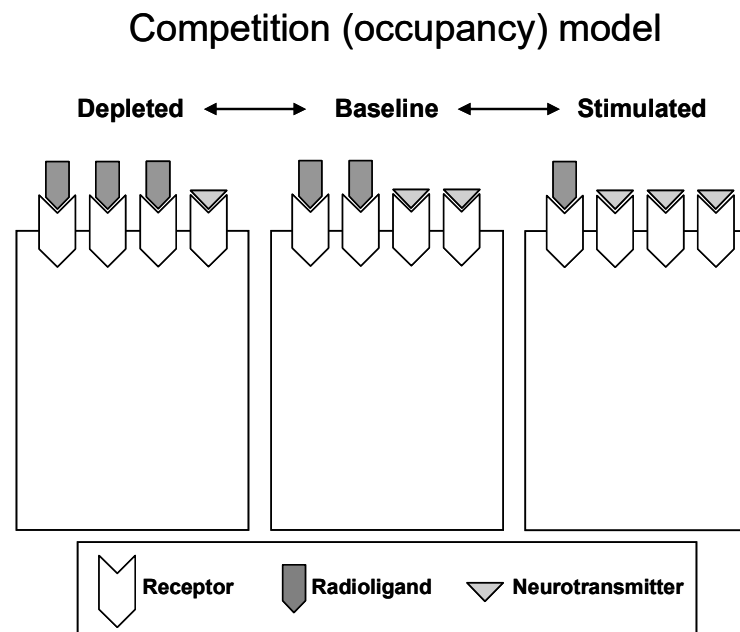
Madsen et al., *Neuropsychopharmacol* 2019

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

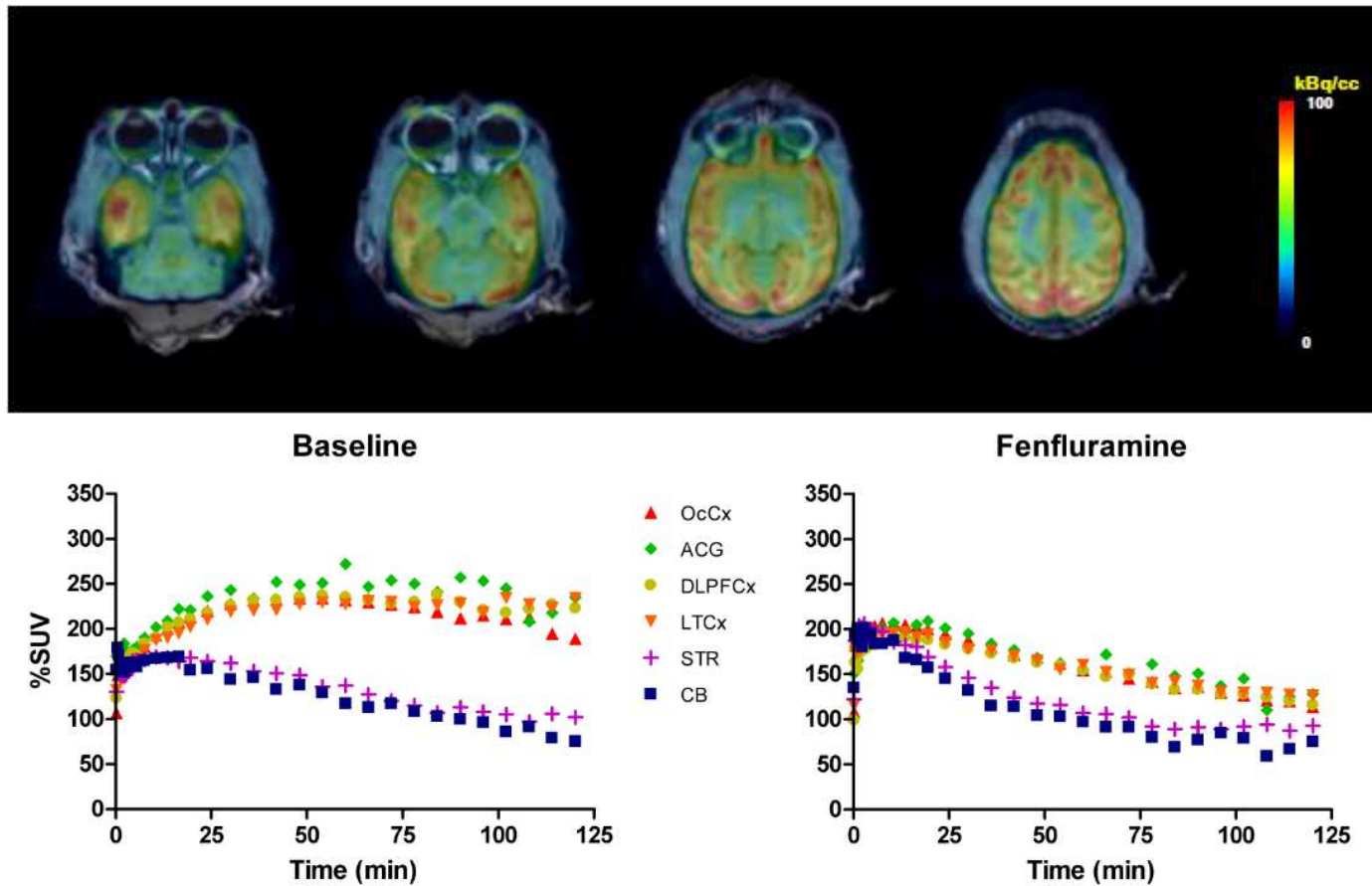


Madsen et al., *Neuropsychopharmacol* 2019

Is binding sensitive to changes in endogenous 5-HT?

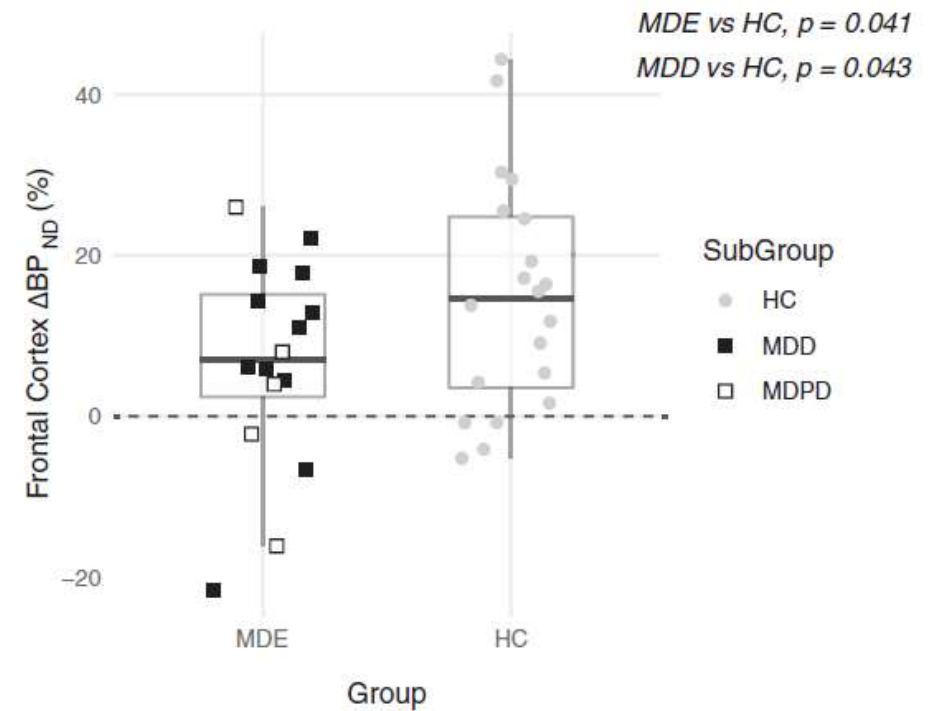
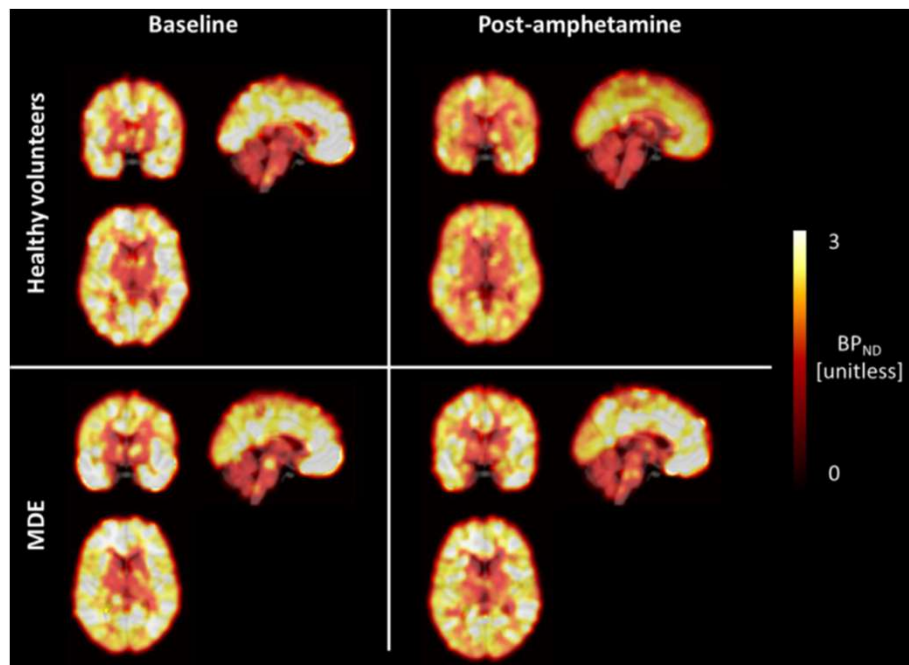


[¹¹C]Cimbi-36 is sensitive to the 5-HT releaser fenfluramine



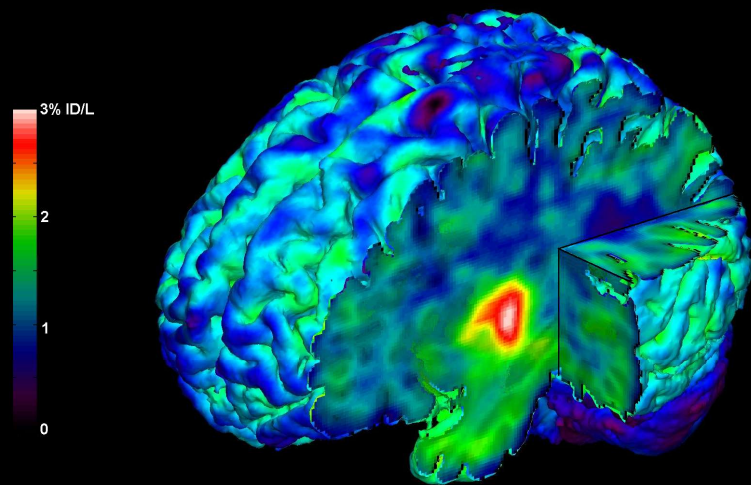
Finnema et al

Change in ^{11}C -Cimbi release after amphetamine stimulation



Erritzøe et al, Biol Psychiatry, 2023

5-HT₄ receptors



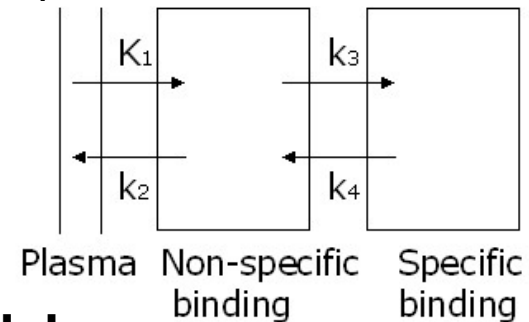
- Subgroup of serotonin receptors
- Located primarily in
 - caudate nucleus (32 pCi/mm²)
 - putamen (26 pCi/mm²)
 - hippocampus
 - CA1 (30 pCi/mm²)
 - Dentate gyrus (17 pCi/mm²)
 - cortical (12-20 pCi/mm²)
 - Cerebellum (<1 pCi/mm²)
- Clinical relevance:
 - Alzheimer's Disease
 - Enhance learning and memory
 - May diminish the deposition of pathological amyloid plaques

Quantification of 5-HT₄ receptors

- (at that time) New PET tracer: ¹¹C-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**

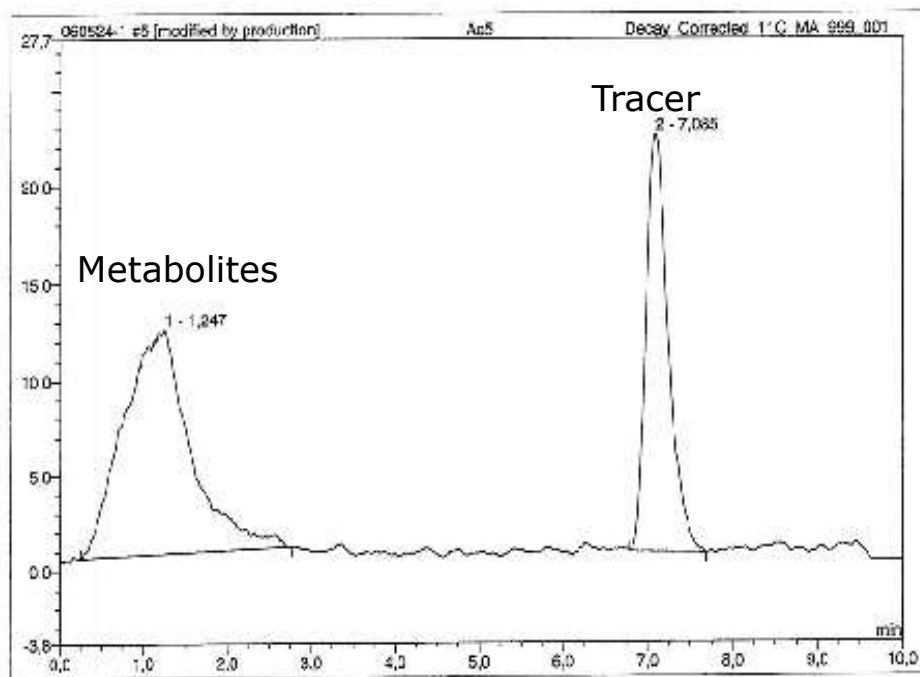
- Based on assumptions
- Non-invasive
- Needs validation

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



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

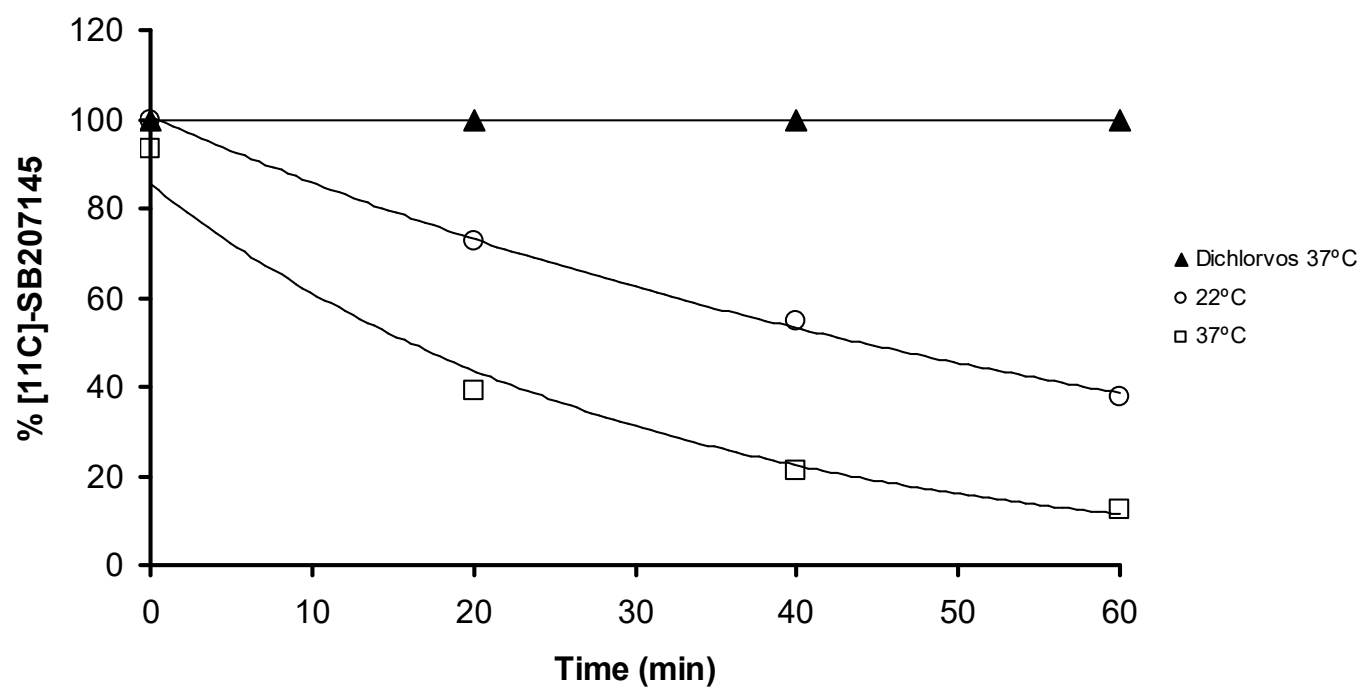
Fraction of unmetabolized

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

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

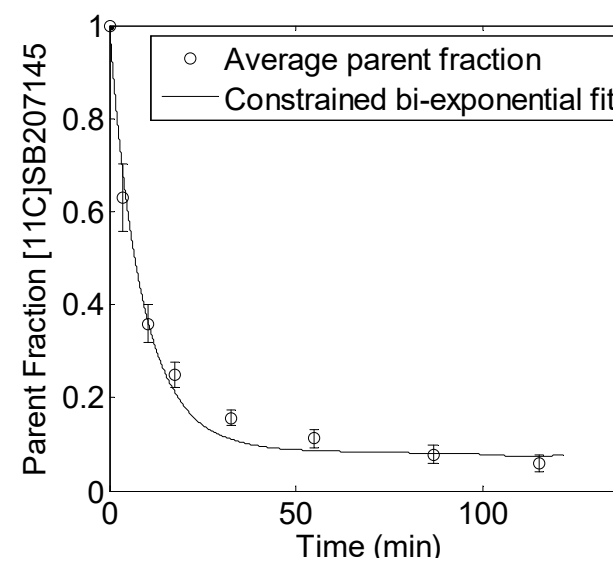
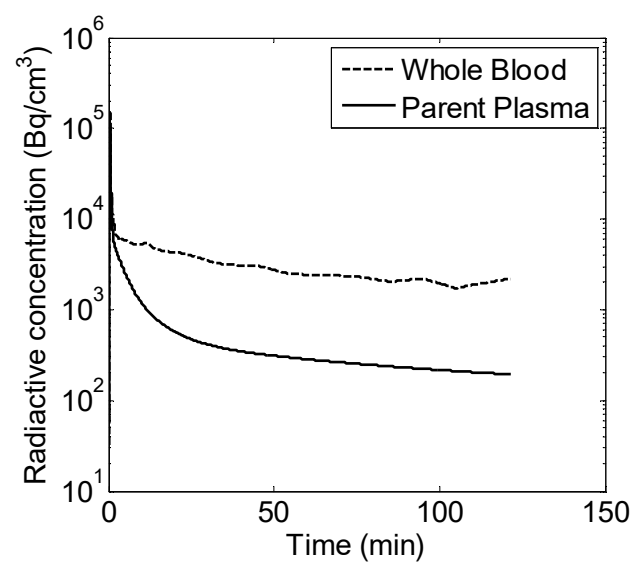
- Adding the esterase
inhibitor dichlorvos to
the blood samples
stops the metabolism
immediately

Compound stability in blood

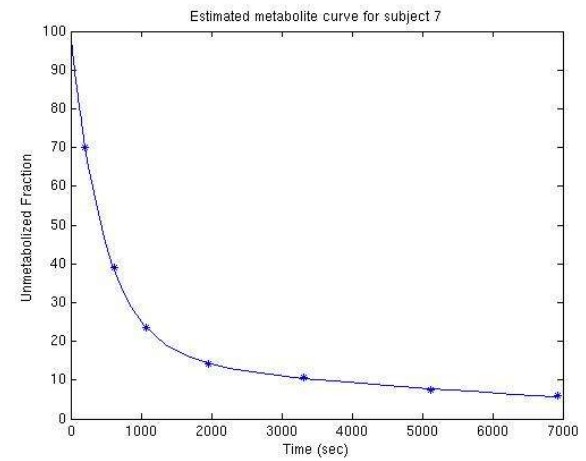
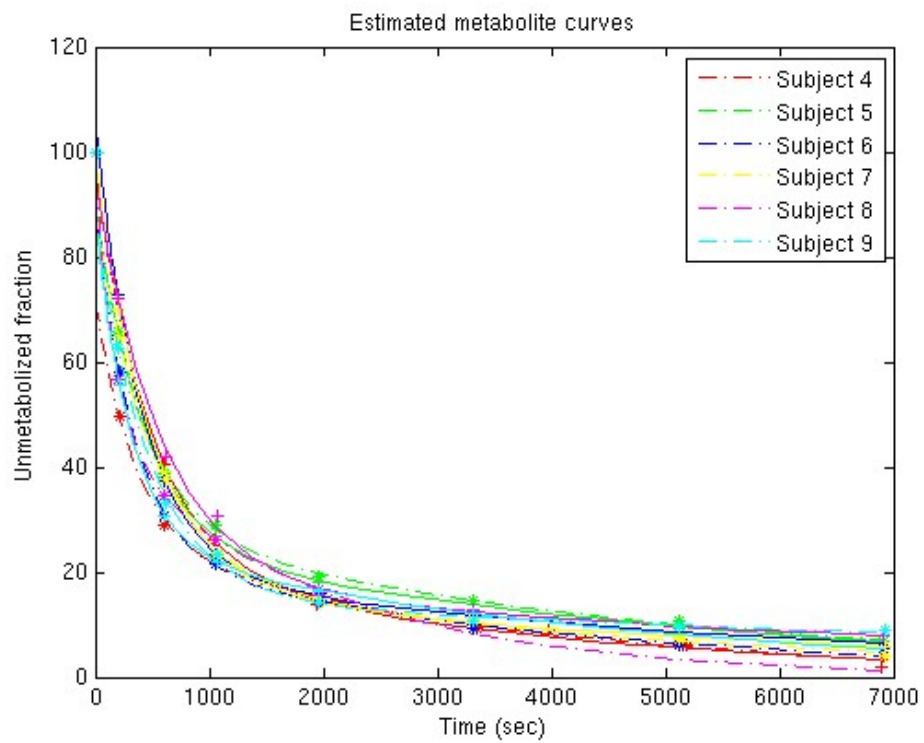


Marner et al, 2009 & 2010

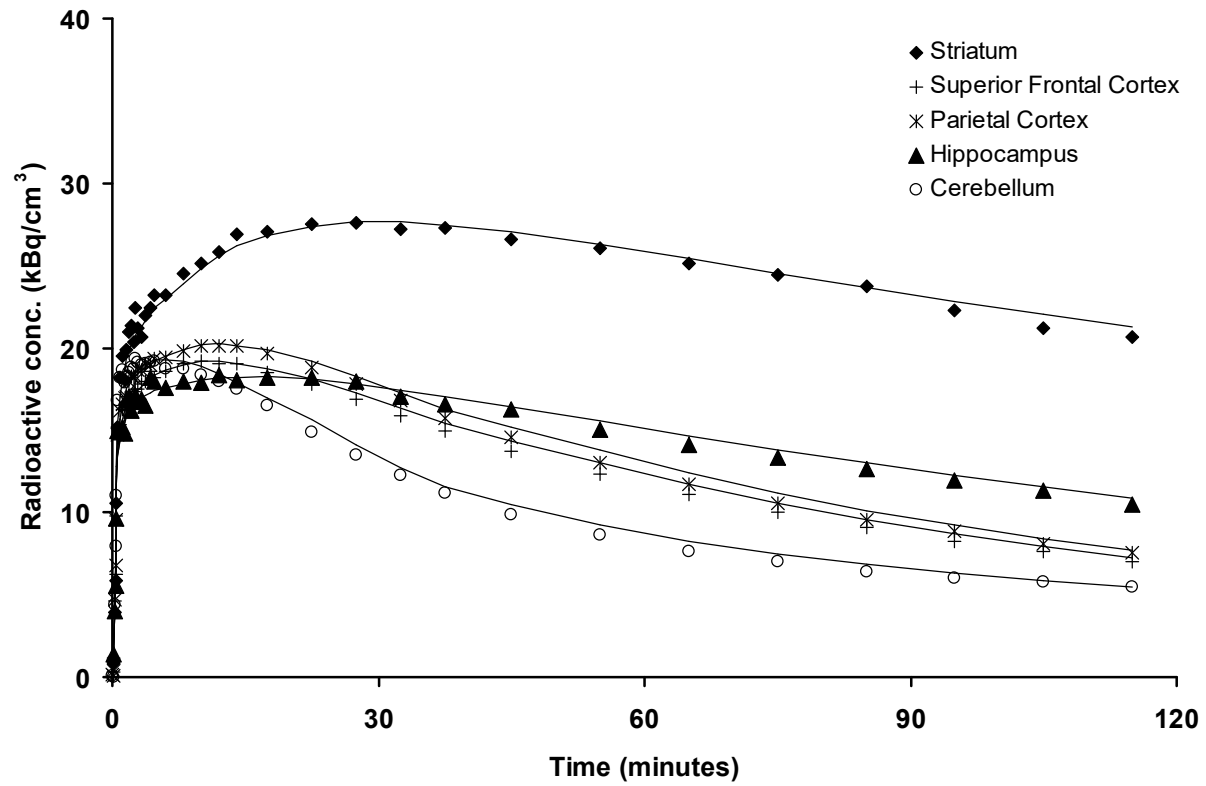
SB207145 systemic metabolism



Metabolite Curves



Time Activity Curves



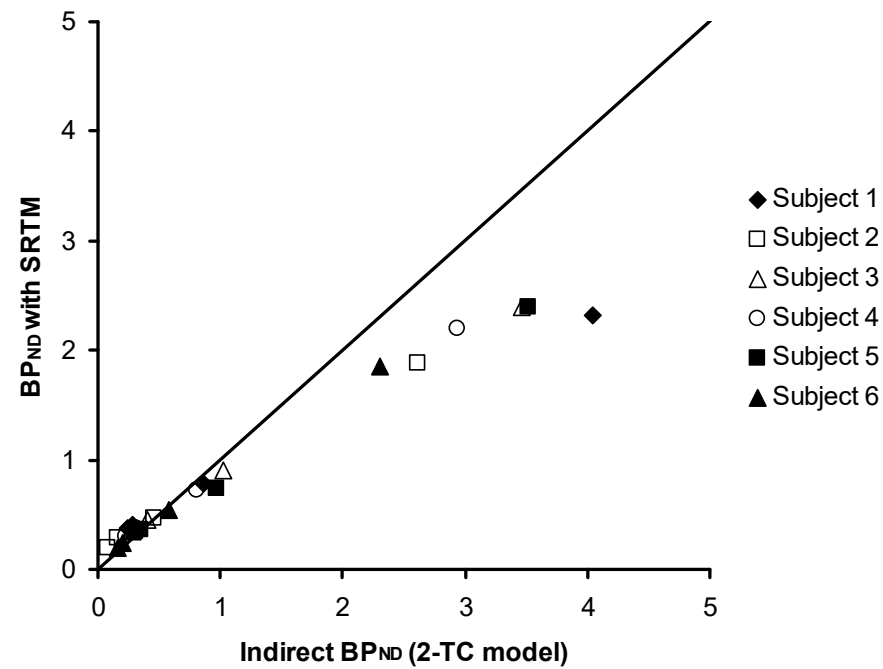
Results: Marked lower variability with the Simplified Reference Tissue Model

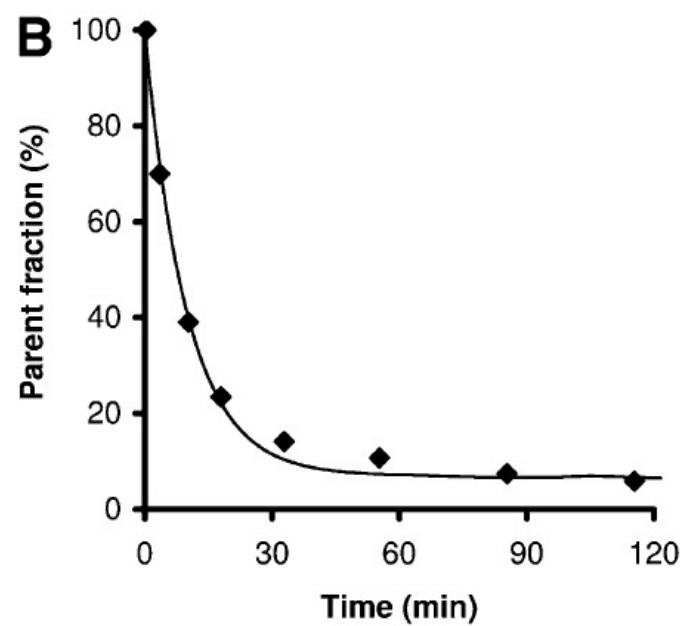
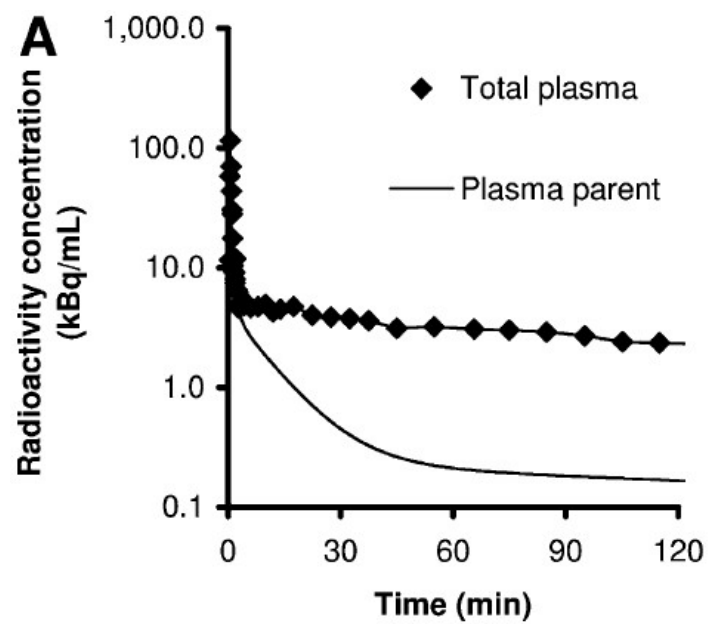
	<i>2- Tissue Compartment Model</i>		<i>Simplified Reference Tissue Model</i>	
Regions	Mean (SD)	Variability in test-retest (SD)	Mean (SD)	Variability in test-retest (SD)
Left caudatus	5.19 (1.03)	0.24 (0.20)	3.24 (0.35)	0.06 (0.04)
Right caudatus	5.22 (1.41)	0.32 (0.18)	3.25 (0.19)	0.04 (0.04)
Left putamen	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)

V_T

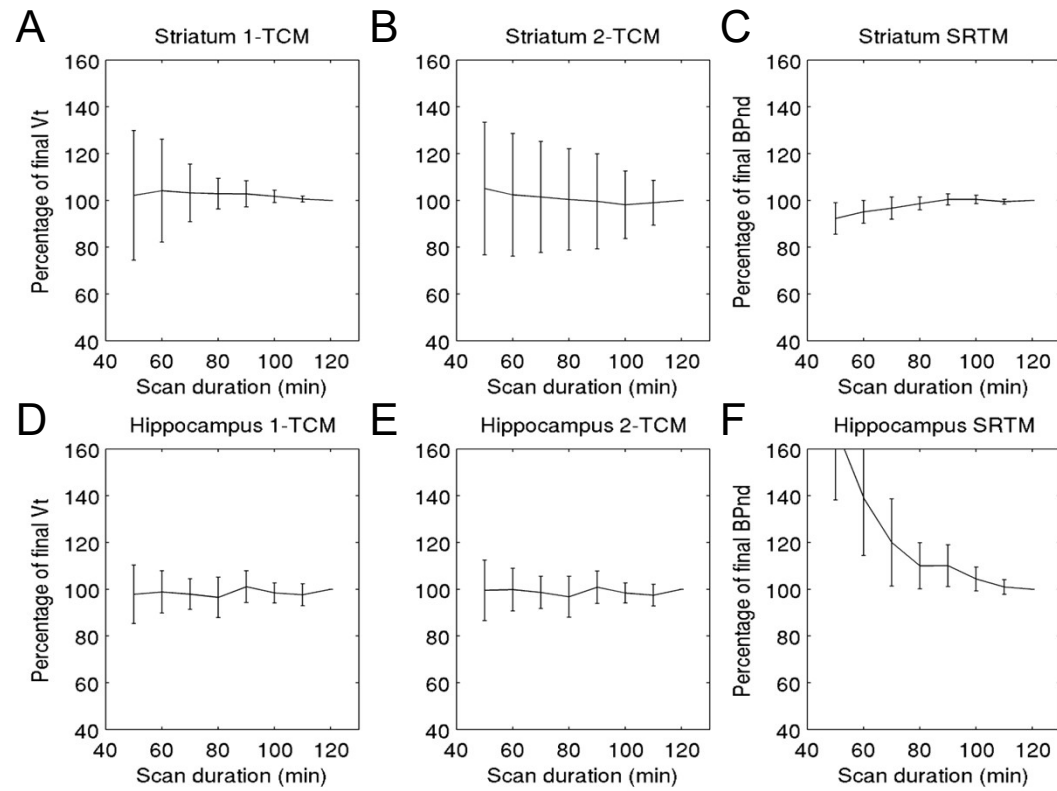
BP_{ND}

Bias in high binding regions with the Simplified Reference Tissue Model

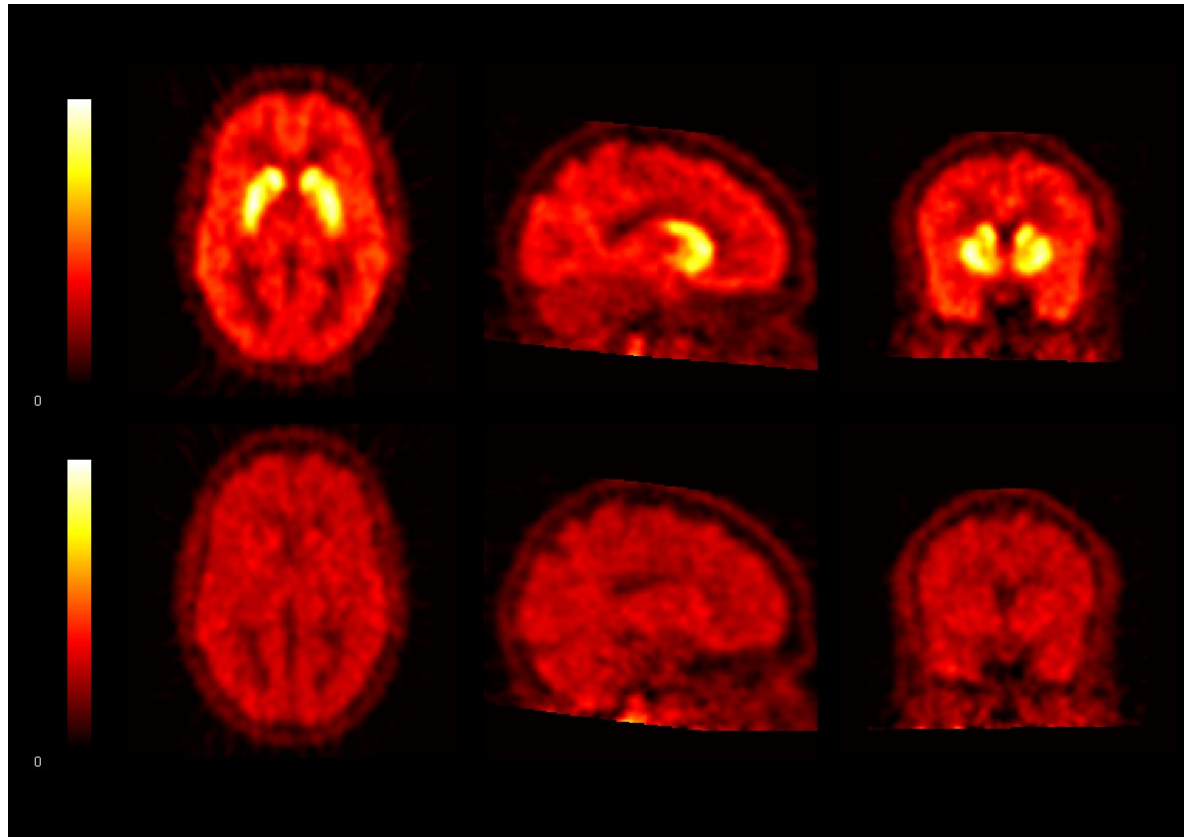




How long do you need to scan?



Blocking of 5-HT₄ receptors

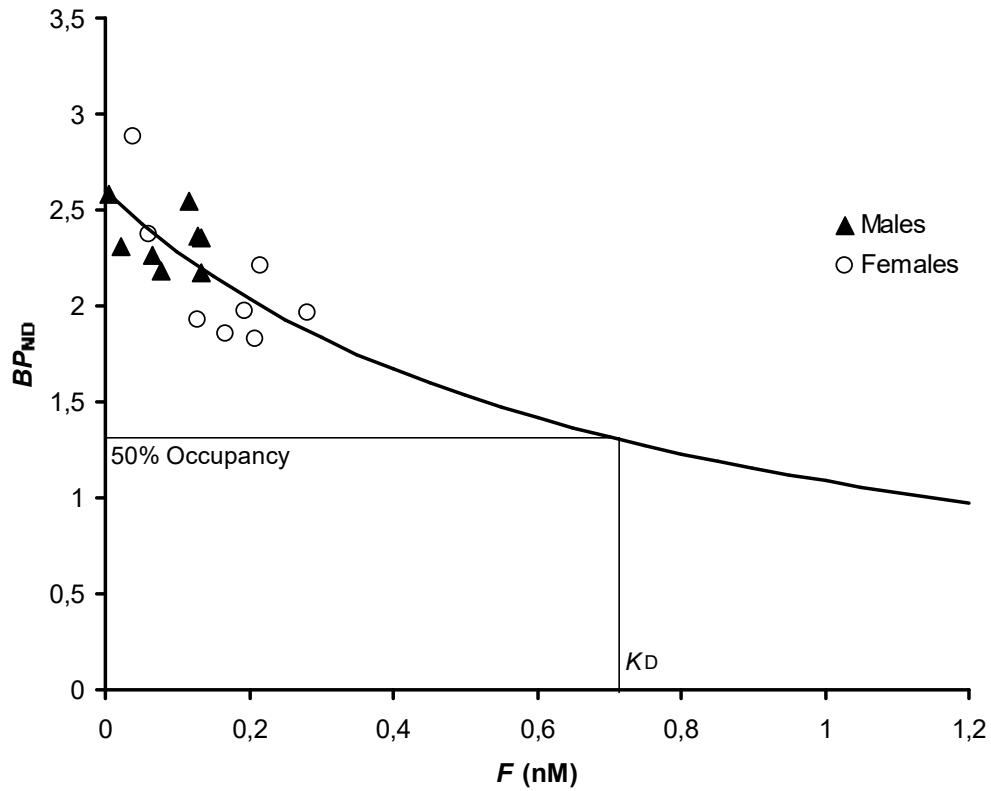


Pretreatment with
a 5-HT₄ receptor
antagonist

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 K_d or B_{max} values

Occupancy – estimation of in vivo K_D



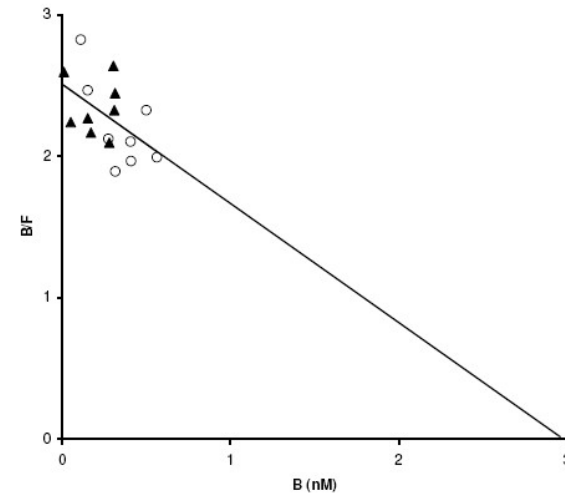
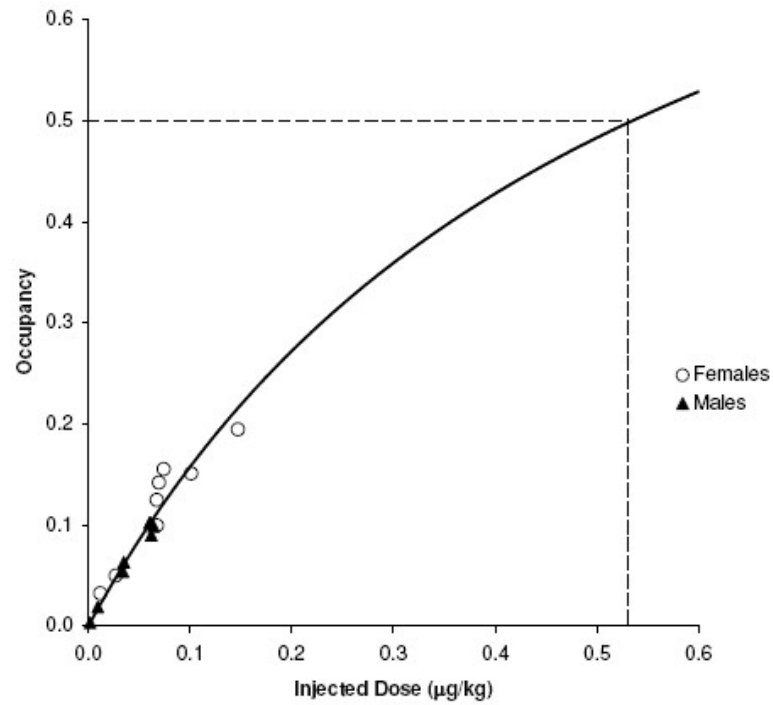
$$B = \frac{B_{\text{avail}} \cdot F}{F + K_D} \Leftrightarrow$$

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

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

Marner et al, 2009

Occupancy



Madsen et al, Nucl Med Biol. 2011

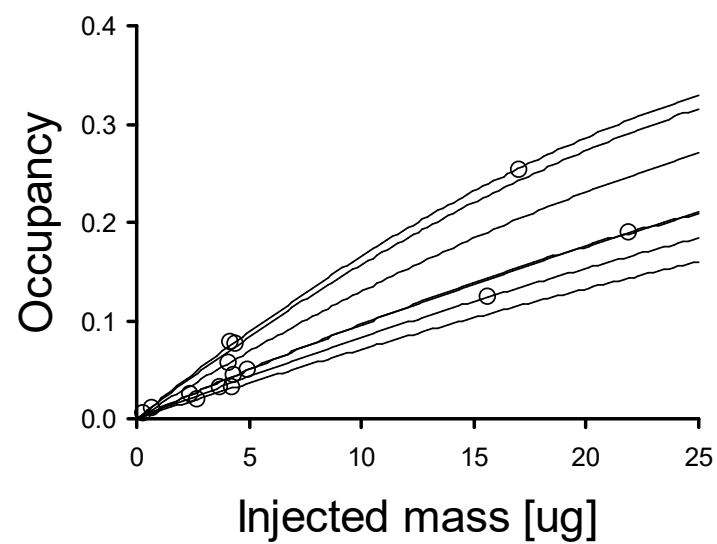
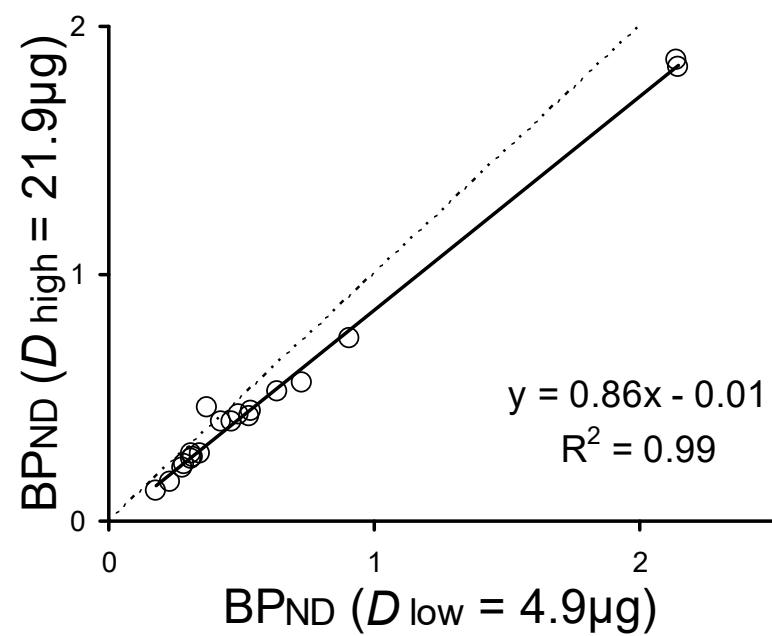
$$B = \frac{B_{\text{Max}} F}{K_{\text{D}} + F}$$

$$\frac{B}{F} = \frac{B_{\text{Max}}}{K_{\text{D}}} = BP$$

$$O = \frac{D}{D + ID_{50}}$$

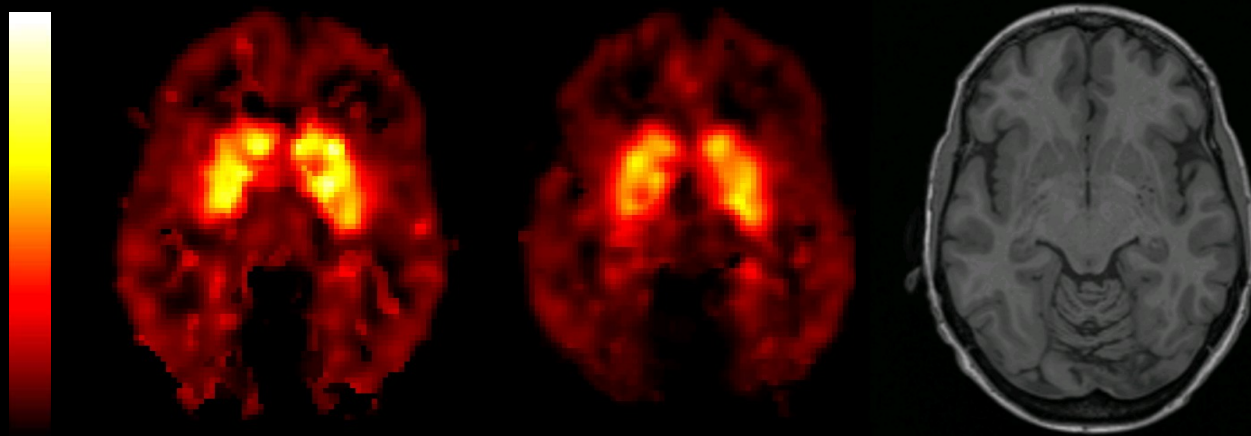
$$BP_{\text{Occupied}} = (1 - O)BP_{\text{Baseline}}$$

$$ID_{50} = \frac{\alpha D_{\text{High}} - D_{\text{Low}}}{1 - \alpha}$$



Madsen et al, Nucl Med Biol. 2011

Mass doses and binding

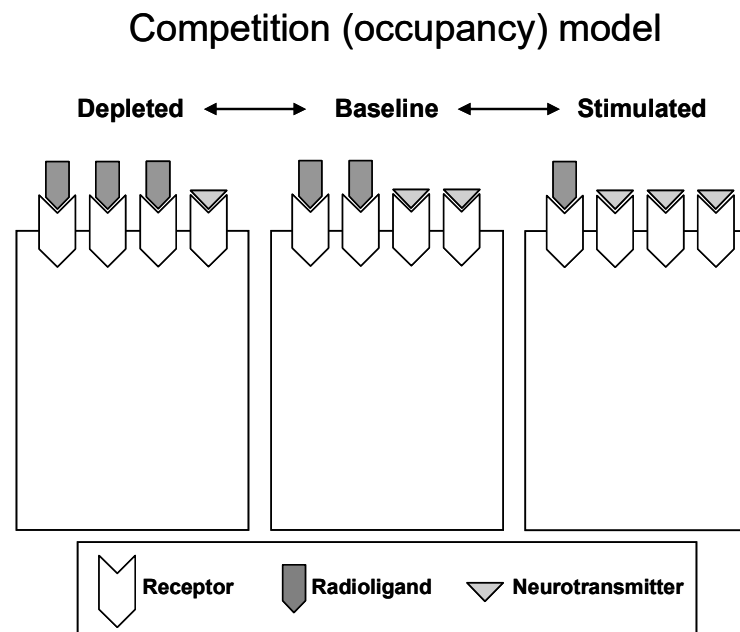


Parametric BP_{ND} images after injection of 4.9 μg ligand (left), 21.9 μg ligand (middle) and the corresponding MRI (right).

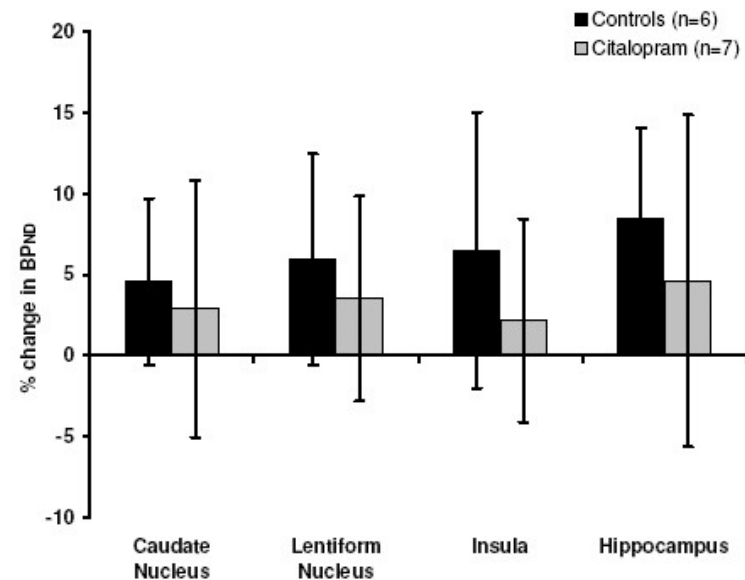
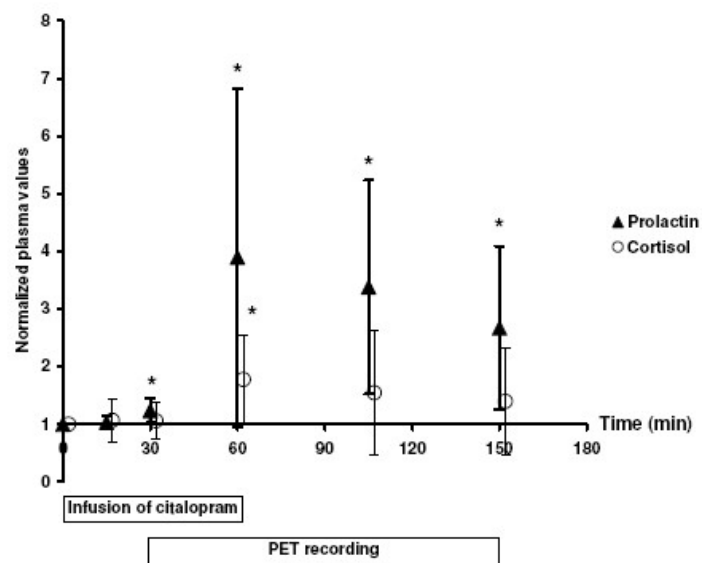
BP_{ND} decreased by 14% as a result of mass dose.

	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 $\pm$ 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

Is binding sensitive to changes in endogenous 5-HT?



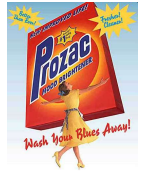
5-HT challenge with citalopram infusion



Marner et al, 2010



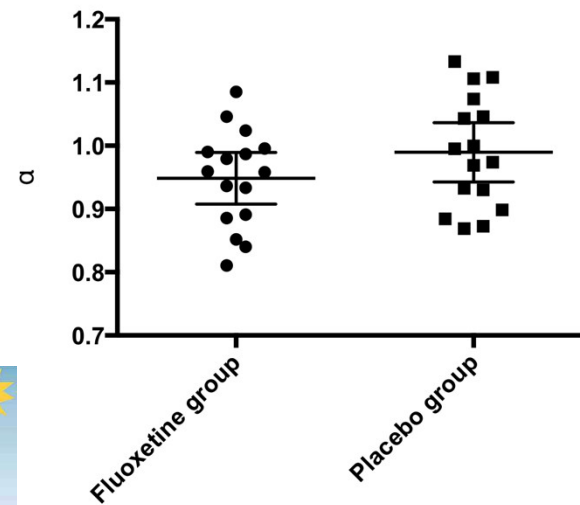
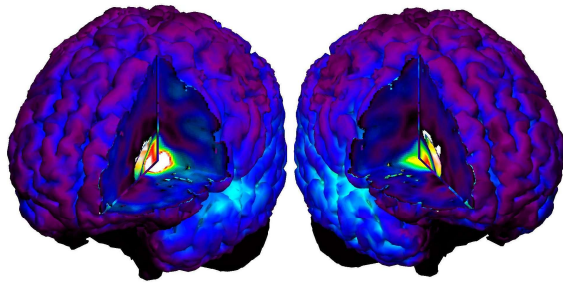
SSRI project: Hypotheses



- 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-HT₄ receptor reflects brain 5-HT levels

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



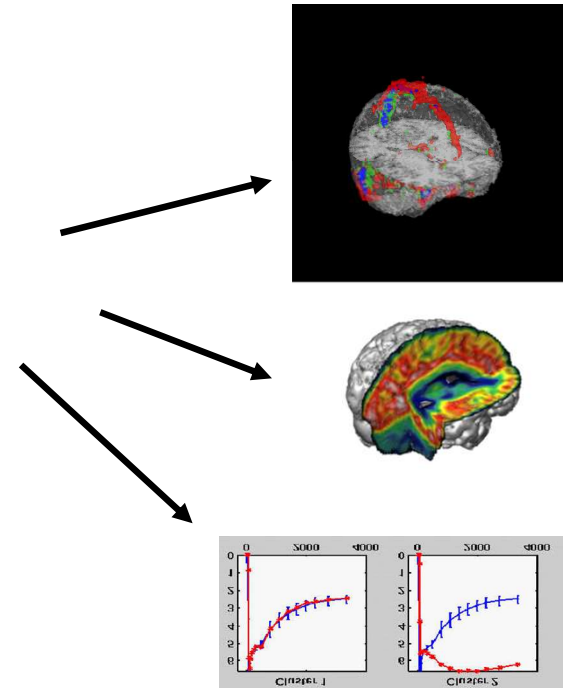
Haahr et al, Mol Psych 2014

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-HT₄ 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 <5μg 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