

Discovery Research

In Vivo Receptor Occupancy Capabilities



Discovery Research
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LC-MS/MS Based Receptor Occupancy

Overview: Suven Publications



- **In vivo receptor occupancy assay of histamine H3 receptor antagonist in rats using non-radiolabeled tracer.** *J Pharmacol Toxicol Methods*. 2012, 65(3):115-121. Nirogi R, Kandikere V, Bhyrapuneni G, Muddana N, Saralaya R, Ponnamaneni RK, Manoharan AK.
- **Rat thalamic $\alpha_4\beta_2$ neuronal nicotinic acetylcholine receptor occupancy assay using LC-MS/MS.** *J Pharmacol Toxicol Methods*. 2012, 65(3):136-141. Nirogi R, Kandikere V, Bhyrapuneni G, Saralaya R, Muddana N, Ajjala DR.
- **Methyllycaconitine: a non-radiolabeled ligand for mapping α_7 neuronal nicotinic acetylcholine receptors - in vivo target localization and biodistribution in rat brain.** *J Pharmacol Toxicol Methods*. 2012, 66(1):22-8.2. Nirogi R, Kandikere V, Bhyrapuneni G, Saralaya R, Muddana N, Komarneni P.
- **In-vivo rat striatal 5-HT4 receptor occupancy using non-radiolabelled SB207145.** *J Pharm Pharmacol*. 2013, 65(5):704-712. Nirogi R, Kandikere V, Bhyrapuneni G, Saralaya R, Ajjala DR, Aleti RR, Rasheed MA.
- **Simultaneous in-vivo receptor occupancy assays for serotonin 1A, 2A, and dopamine 2 receptors with the use of non-radiolabelled tracers: Proposed method in screening antipsychotics.** *J Pharmacol Toxicol Methods*. 2017, 85:22-28. Thentu JB, Nirogi R, Bhyrapuneni G, Ajjala DR, Aleti RR, Palacharla RC.

LC-MS/MS or LSA Based Receptor Occupancy

Overview: Methodology



LC-MS/MS based

Formulation

Test compound dosing
p.o./s.c./i.p./i.v.

Animal model: rat/ mouse/
guinea pig



Liquid scintillation analyzer (LSA) based

Receptor
occupancy
and
exposure
correlation

Non-radiolabelled tracer (*i.v.*) dose at T_{max} of test compound

Cervical dislocation & trunk blood collection

Brain regional isolation

Sample homogenation, protein precipitation & centrifugation

LC-MS/MS quantification of tracer/ test compound (ng/g or ng/mL) - API 6500 Q Trap

Radiolabelled ($[^3H]$) tracer (*i.v.*) dose at T_{max} of test compound

Cervical dislocation

Brain regional isolation

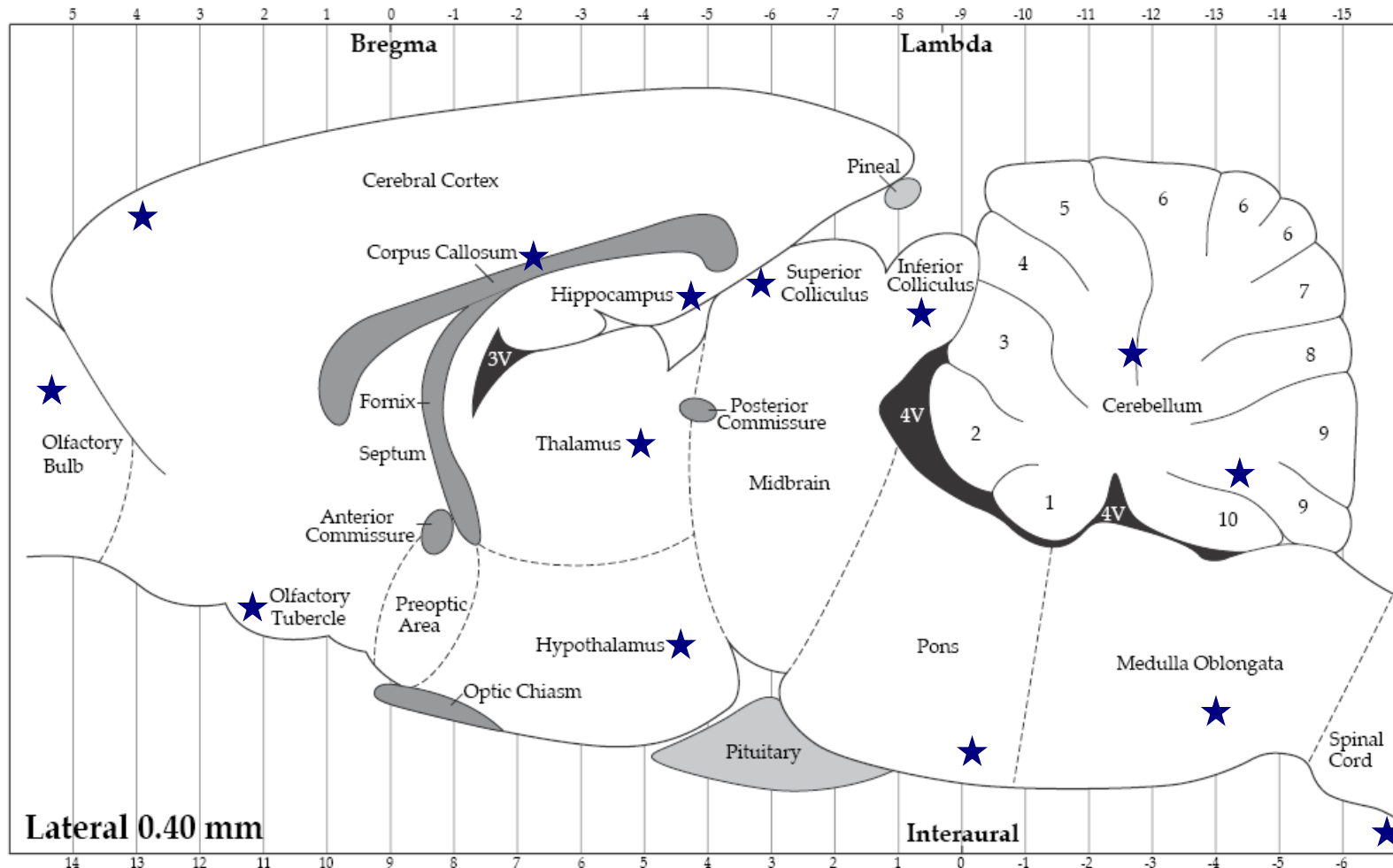
Sample homogenation & filtration or tissue digestion

Radioactivity determination by Liquid scintillation counter -Tricarb 3110TR (CPM or DPM)

Receptor occupancy calculation
(specific binding/ positive control method)

LC-MS/MS Based Receptor Occupancy

Overview: Brain Regions



★
Currently practiced
rat brain regions at
Seven

LC-MS/MS Based Receptor Occupancy

Overview: Validated Targets



Receptor	Tracer	Receptor	Tracer	Receptor	Tracer
#, * 5-HT _{1A}	WAY-100635■	A _{2A}	SCH442416	Sigma 1	FTC-146
5-HT _{1B}	AZ10419369	nAChR α ₄ β ₂	ZW-104	MAO-A	Harmine
* 5-HT _{2A}	MDL-100907	nAChR α ₇	Methyllycaconitine	MAO-B	Selegiline
5-HT _{2C}	SB242084	Histamine H ₃	GSK-189254		
5-HT ₄	SB207145	PDE10	AMG 7980		
5-HT ₆	Lu AE60157■	D ₁	SCH39166		
NET	S,S-MeNER	* D ₂	Raclopride■		
DAT	WIN 35428	NK1	GR205171		
#SERT	DASB	mGluR5	mPEPy		
GABA _A	Flumazenil	Adrenergic α _{1A}	Prazosin		
CB ₁	AM251				

All tracer are non-radiolabelled tracers; ■ Radiolabelled tracers; *# Dual or triple target receptor occupancy assay

5-HT: Serotonin; NERT: Nor epinephrine reuptake transporter; DAT: Dopamine reuptake transporter; SERT: Serotonin reuptake transporter; GABA: Gamma amino butyric acid; CB: Cannabinoid; A: Adenosine; nAChR: nicotinic acetylcholine receptor; PDE: Phosphodiesterase; D: Dopamine; NK: Neurokinin; mGluR: metabotropic glutamate receptor, MAO: monoamine oxidase

LC-MS/MS Based Receptor Occupancy

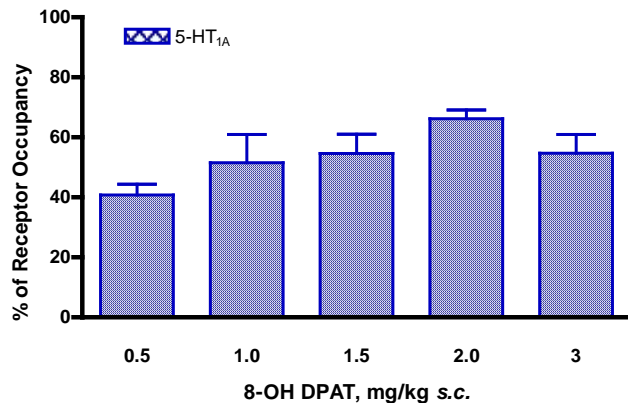
5-HT_{1A} in Rats



Specific region : Frontal Cortex Non specific region: Cerebellum Tracer: WAY 100635

8-OHDPAT

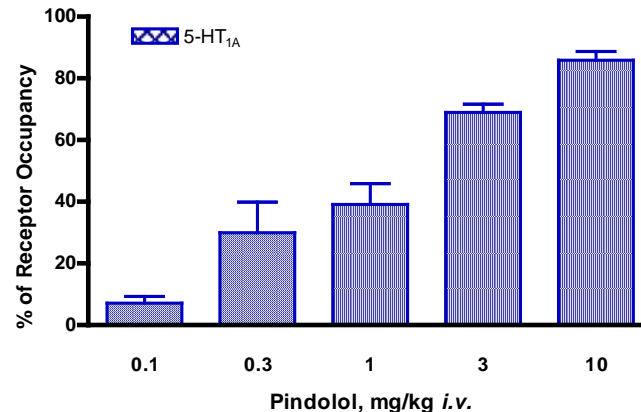
Ki = 0.58 nM



SUVEN ED₅₀ = 0.95 mg/kg, s.c.
Reported ED₅₀ = 0.60 mg/kg, s.c.

Pindolol

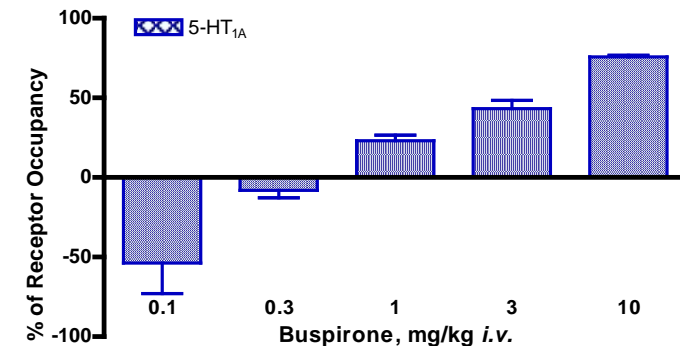
Ki = 14.4 nM



SUVEN ED₅₀ = 1.27 mg/kg, i.v.
Reported ED₅₀ = 0.44 mg/kg, i.v.

Buspirone

Ki = 8.9 nM



SUVEN ED₅₀ = 3.74 mg/kg, i.v.
Reported ED₅₀ = 5.00 mg/kg, i.v.

Synapse., 2000; Psychopharmacology., 1997; Neuropsychopharmacology., 2004; Nuclear Medicine & Biology., 2000.

The calculated % receptor occupancy ED₅₀ value from non-radiolabeled tracer is comparable to reported ED₅₀ from study measured using radiolabeled tracer

LC-MS/MS Based Receptor Occupancy

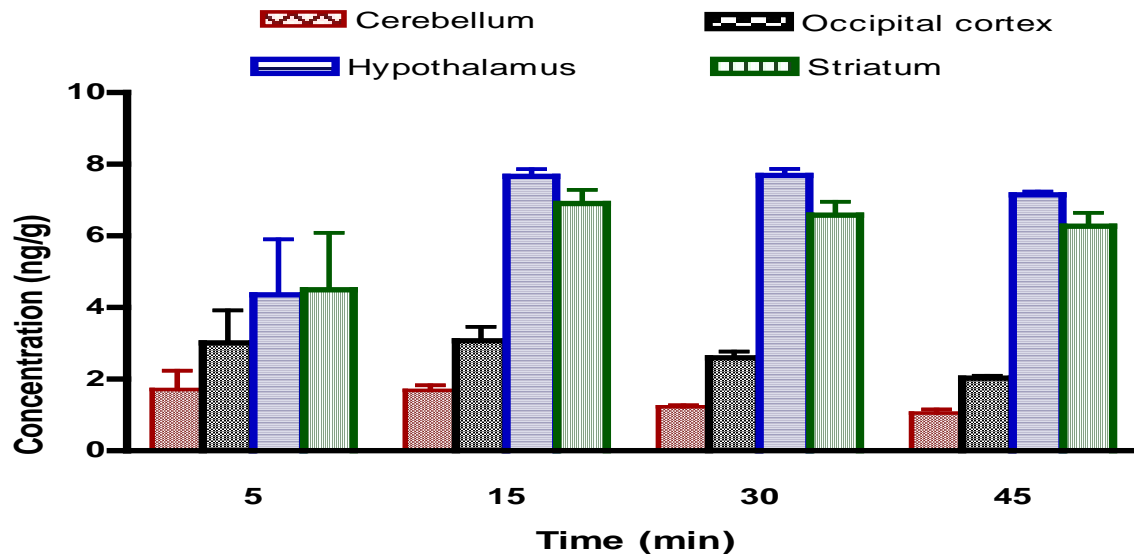
5-HT_{1B} in Guinea Pigs



Specific region: Hypothalamus

Non specific region: Cerebellum

Tracer: AZ10419369



AZ10419369, shown high specific binding in hypothalamus and striatal regions and cerebellum as non specific region. This tracer can be employed in screening the ligands specific to 5-HT_{1B} receptor using ratio method.

Maier et al. *The Journal Of Pharmacology and Experimental Therapeutics*, 2009, Vol. 330



LC-MS/MS Based Receptor Occupancy

5-HT_{2A} in Rats

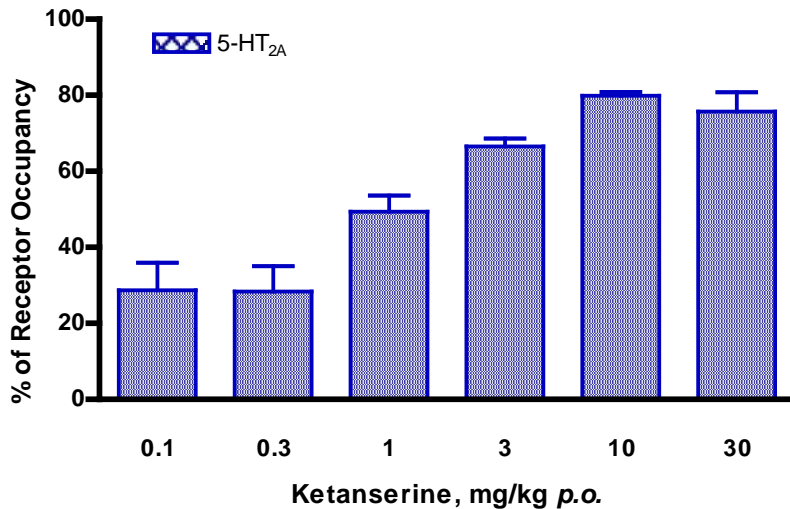
Specific region: Frontal cortex

Non specific region: Cerebellum

Tracer: MDL 100907

Ketanserin

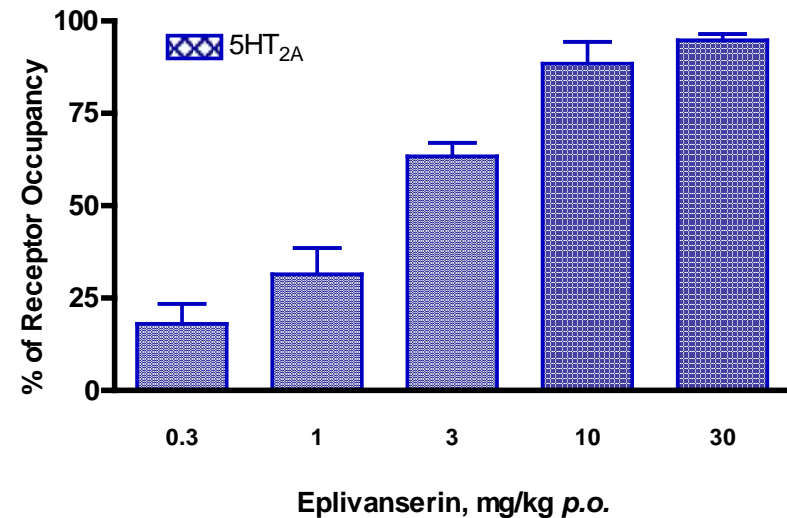
K_i = 3 nM



SUVEN ED₅₀ = 1.04 mg/kg, p.o.
Reported ED₅₀ = 0.32 mg/kg, p.o.

Eplivanserin

K_i = 0.12 nM



SUVEN ED₅₀ = 1.79 mg/kg, p.o.
Reported ED₅₀ = 1.50 mg/kg, p.o.

Life Science., 2007.

The calculated % receptor occupancy ED₅₀ value from non-radiolabeled tracer is comparable to reported ED₅₀ from study measured using radiolabeled tracer

LC-MS/MS Based Receptor Occupancy

5-HT_{2C} in Rats



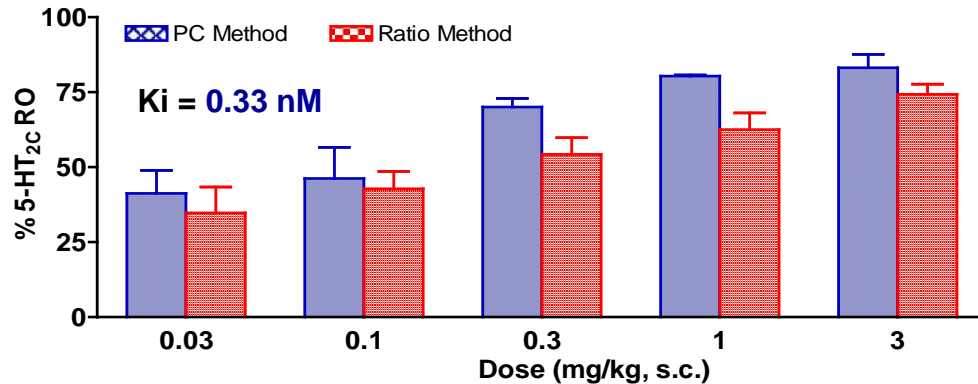
Specific region: Choroid plexus

Non specific region: Cerebellum

Tracer: SB 242084

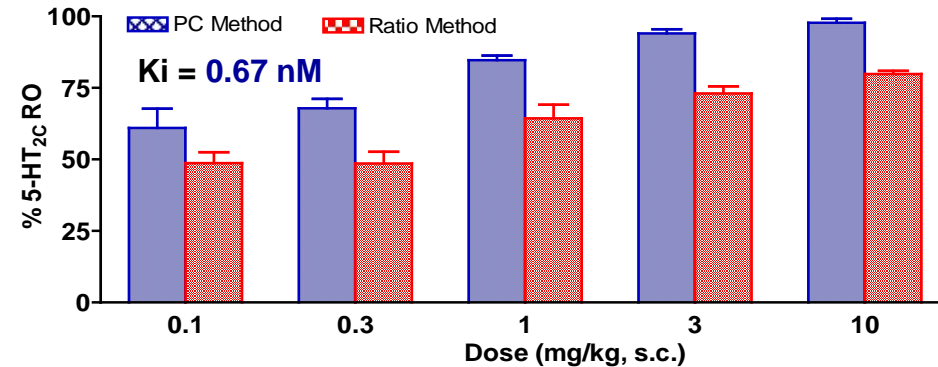
Ritanserin

SUVEN ED₅₀ = 0.08 mg/kg, s.c.



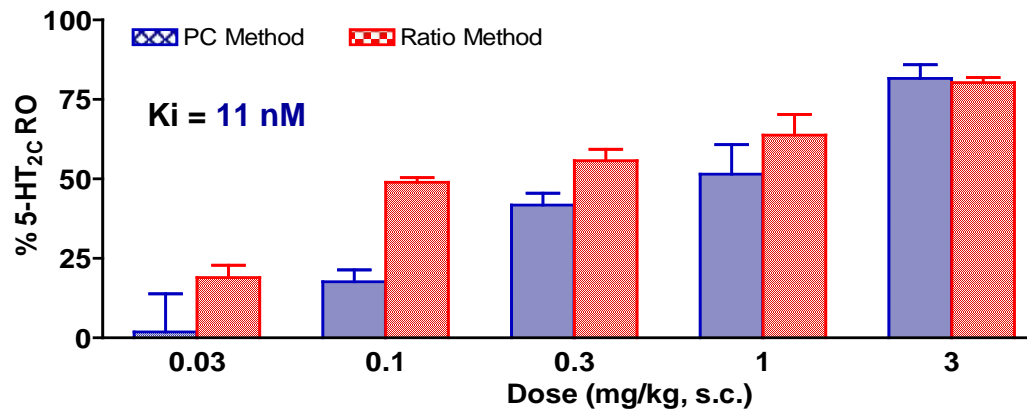
Mianserin

SUVEN ED₅₀ = 0.04 mg/kg, s.c.



Olanzapine

SUVEN ED₅₀ = 0.68 mg/kg, s.c.



LC-MS/MS Based Receptor Occupancy

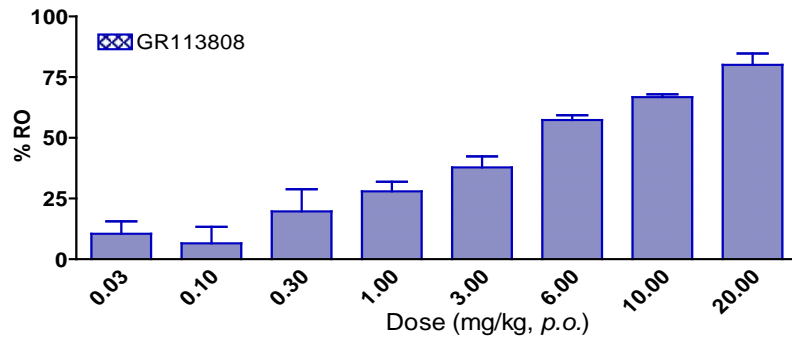
5-HT₄ in Rats



Specific region: Striatum

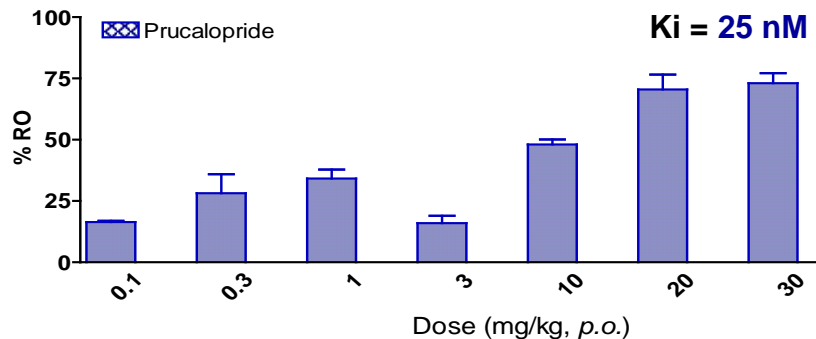
GR113808 (Antagonist)

Ki = 0.3 nM



Prucalopride (Agonist)

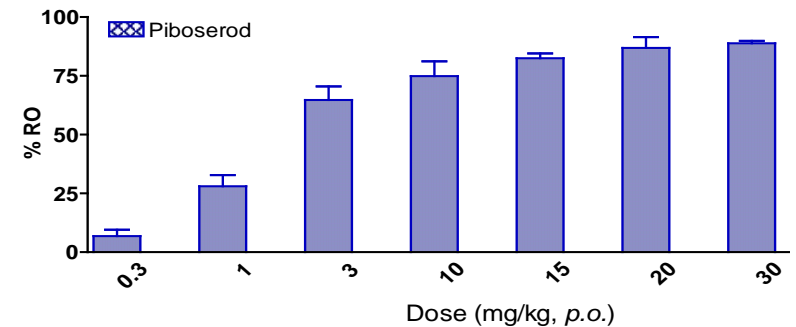
Ki = 25 nM



Non specific region: Cerebellum

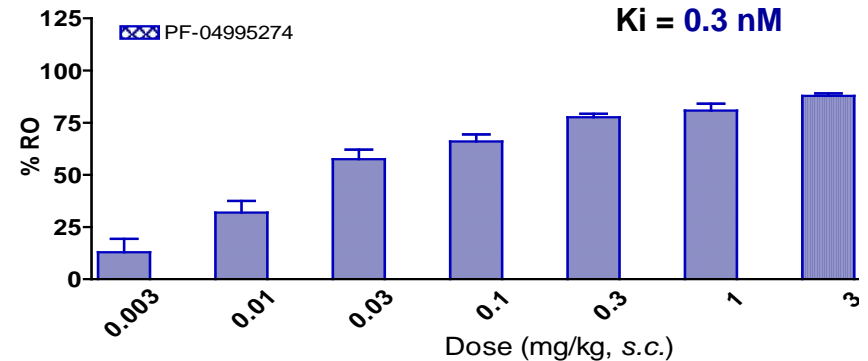
Piboserod (Antagonist)

Ki = 0.12 nM



PF04995274 (Partial agonist)

Ki = 0.3 nM



Reported ID₅₀ = 0.008 mg/kg, s.c.

Obtained ED₅₀ = 0.017 mg/kg, s.c.

Grimwood et al., Pfizer – Poster No: 11818 - Translational receptor occupancy for the 5-HT₄ partial agonist PF-04995274 in rats, non-human primates and healthy volunteers – ICAD-2011 - Paris

LC-MS/MS Based Receptor Occupancy

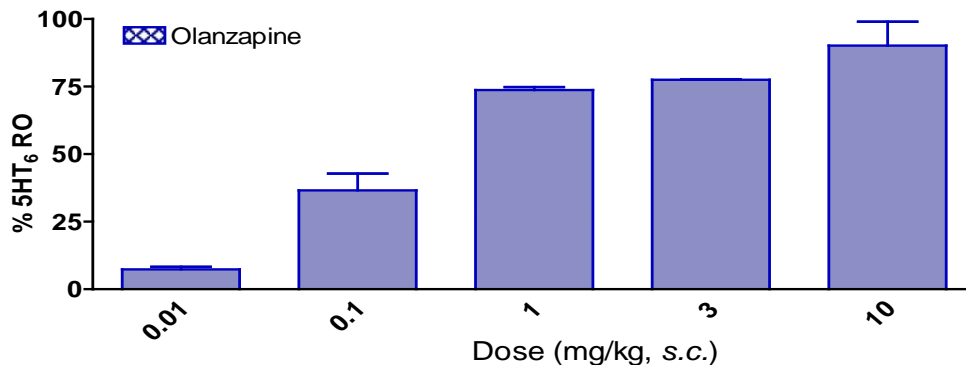
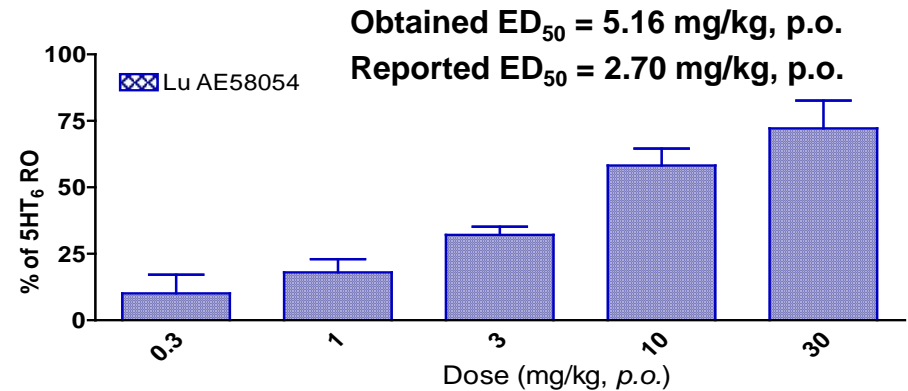
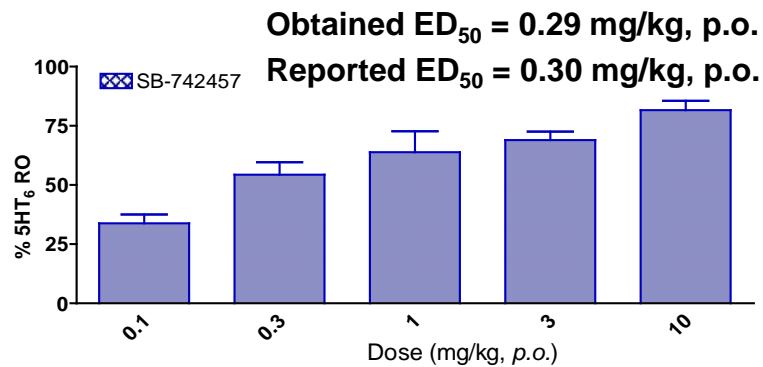
5-HT₆ in Rats



Specific region: Striatum

Non specific region: Cerebellum

Tracer: LuAE-60157



Obtained ED₅₀ = 0.01 mg/kg, s.c.
Reported ED₅₀ = 0.04 mg/kg, s.c.

Jorn Arnt et al., Lu AE58054 a 5-HT₆ antagonist, reverses cognitive impairment induced by subchronic phencyclidine in a novel object recognition test in rats. *International Journal of Neuropsychopharmacology* 2010; 1-13.

Idris et al., Sertindole improves sub-chronic PCP-induced reversal learning and episodic memory deficits in rodents. *Psychopharmacology* 2010; 23-36.

L. Witten et al. *European Journal of Pharmacology* 676 (2012) 6-11

LC-MS/MS Based Receptor Occupancy

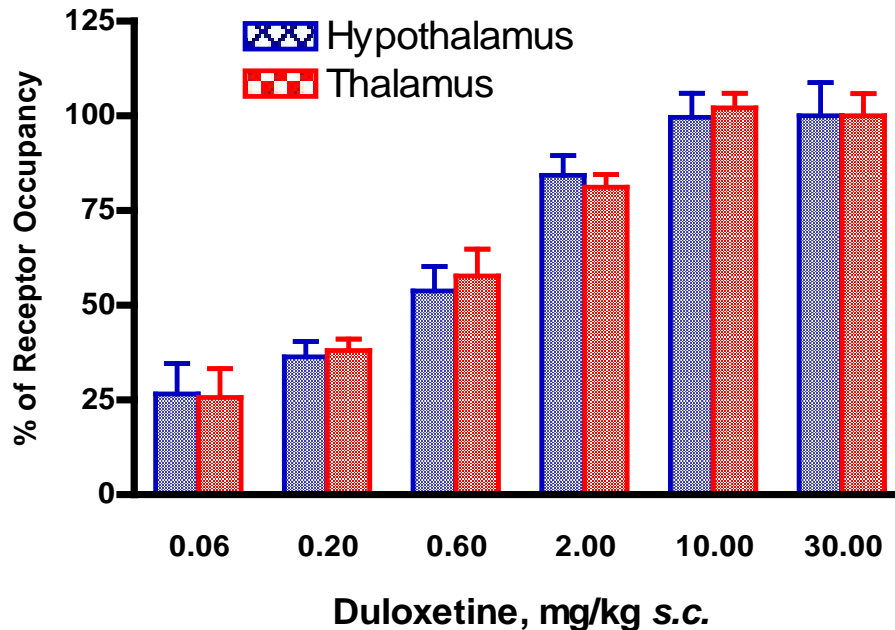
NET in Rats



Specific region: Thalamus/Hypothalamus

Duloxetine

Ki = 10 nM



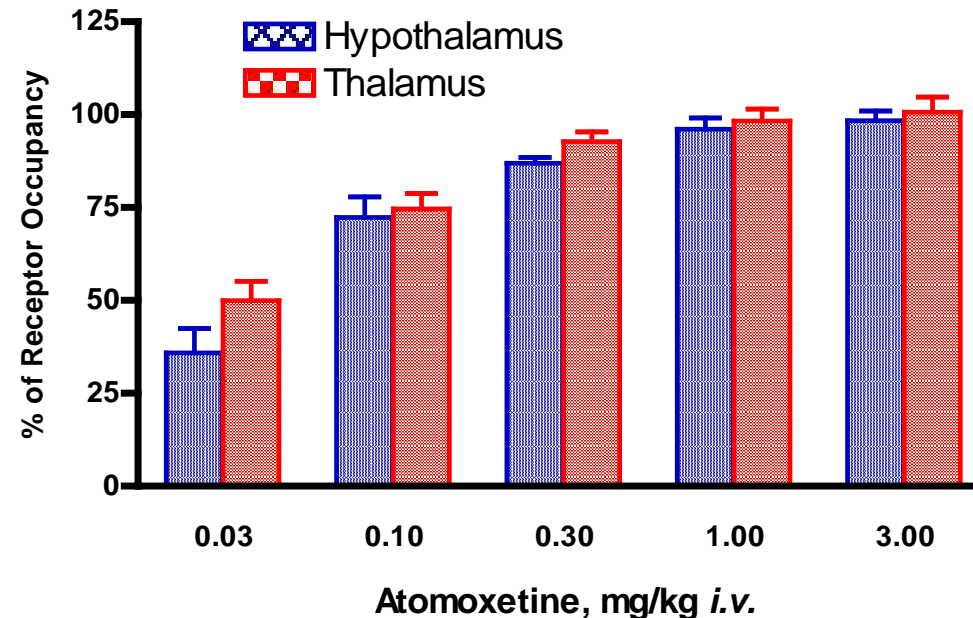
SUVEN ED₅₀ = 0.40 mg/kg, s.c.
Reported ED₅₀ = 1.00 mg/kg, s.c.

Neuropharmacology., 2008 and Eur J Nucl Med Mol Imaging., 2009

Tracer: S,S-MeNER

Atomoxetine

Ki = 5 nM



SUVEN ED₅₀ = 0.04 mg/kg, i.v.
Reported ED₅₀ = 0.028 mg/kg/h or 0.13 mg/kg, i.v.

The calculated % receptor occupancy ED₅₀ value from non-radiolabeled tracer is comparable to reported ED₅₀ from study measured using radiolabeled tracer

LC-MS/MS Based Receptor Occupancy

DAT in Rats

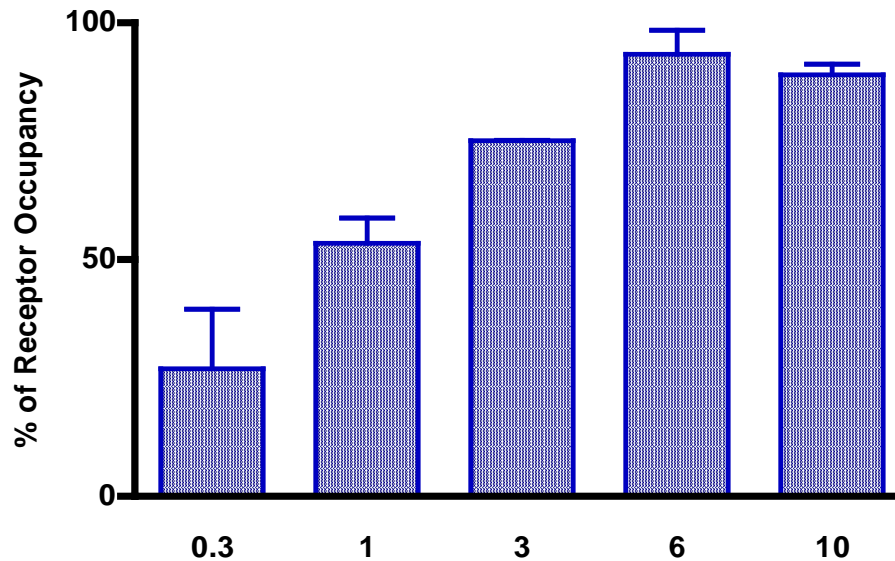


Specific region: Striatum

Non specific region: Cerebellum

Tracer: WIN 35428

GR12909

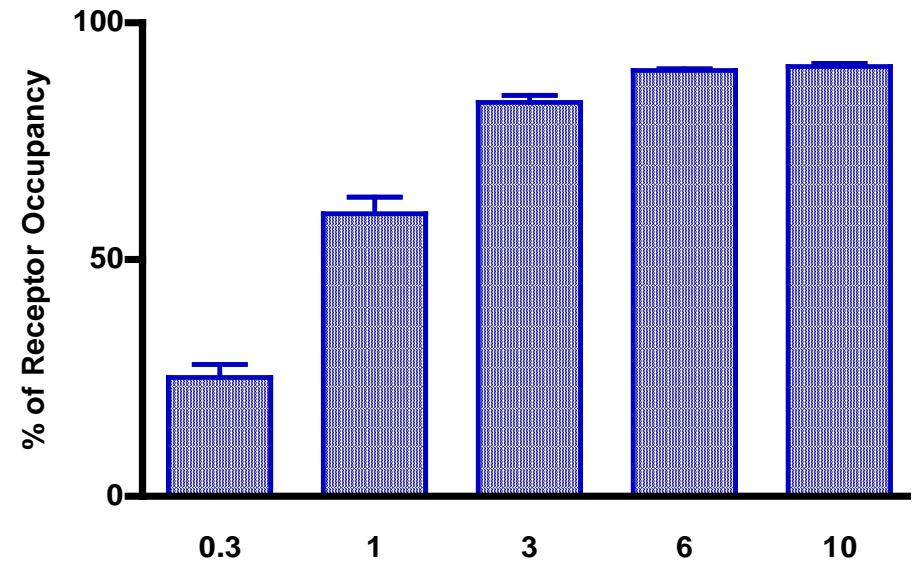


GR12909, mg/kg i.v.

SUVEN ED₅₀ = 0.89 mg/kg, i.v.

Reported ED₅₀ = 1.49 mg/kg, i.v.

Nomifensine



Nomifensine, mg/kg i.v.

SUVEN ED₅₀ = 0.65 mg/kg, i.v.

Reported ED₅₀ = 2.2 mg/kg, i.v.

Scheffel et al. The Journal of Pharmacology and experimental Therapeutics Vol. 257, No. 3, 1991

LC-MS/MS Based Receptor Occupancy

SERT in Rats

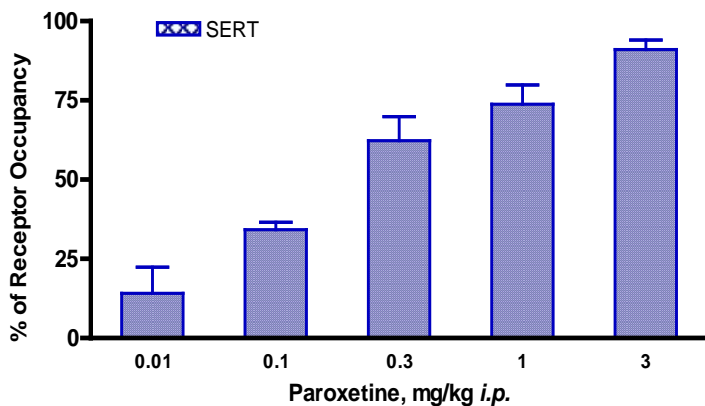


Specific region: Frontal cortex

Tracer: DASB

Paroxetine

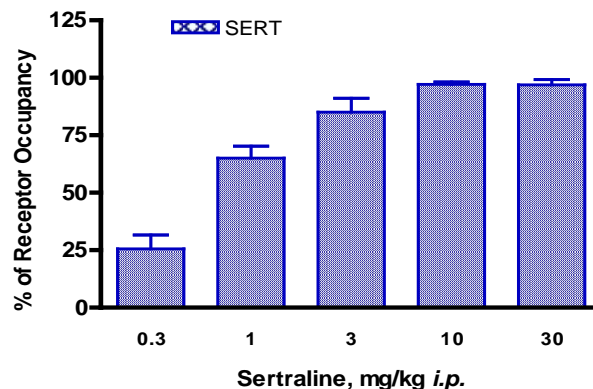
Ki = 0.16 nM



SUVEN ED₅₀ = 1.27 mg/kg, i.p.
Reported ED₅₀ = 1.00 mg/kg, i.p.

Sertraline

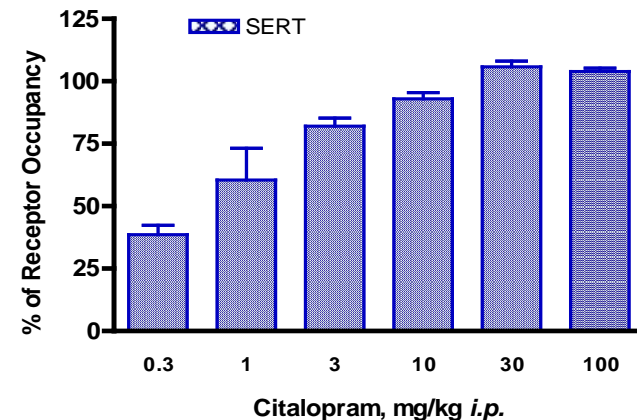
Ki = 0.7 nM



SUVEN ED₅₀ = 1.97 mg/kg, i.p.
Reported ED₅₀ = 2.60 mg/kg, i.p.

Citalopram

Ki = 0.8 nM



SUVEN ED₅₀ = 2.48 mg/kg, i.p.
Reported ED₅₀ = 4.70 mg/kg, i.p.

Synapse., 2000; Psychopharmacology., 1997; Neuropsychopharmacology., 2004; Nuclear Medicine & Biology., 2000.

The calculated % receptor occupancy ED₅₀ value from non-radiolabeled tracer is comparable to reported ED₅₀ from study measured using radiolabeled tracer



LC-MS/MS Based Receptor Occupancy

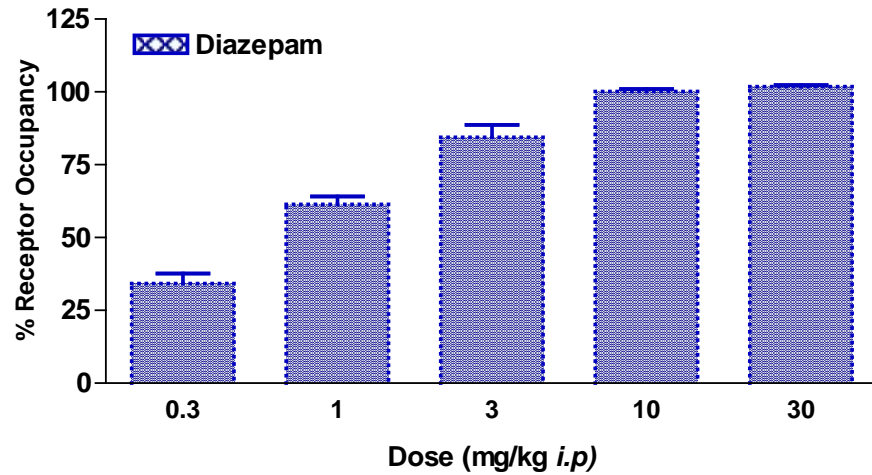
Benzodiazepine GABA_A in Rats

Specific region : Frontal cortex

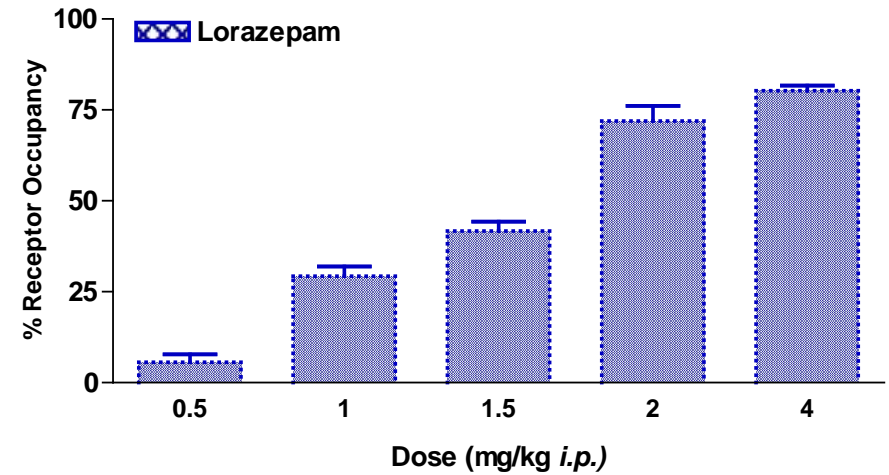
Non specific region: Pons

Tracer: Flumazenil

Diazepam



Lorazepam



JPET., 2007

The calculated % receptor occupancy ED₅₀ value from non-radiolabeled tracer is comparable to reported ED₅₀ from study measured using radiolabeled tracer.

LC-MS/MS Based Receptor Occupancy Cannabinoid-1 in Rats

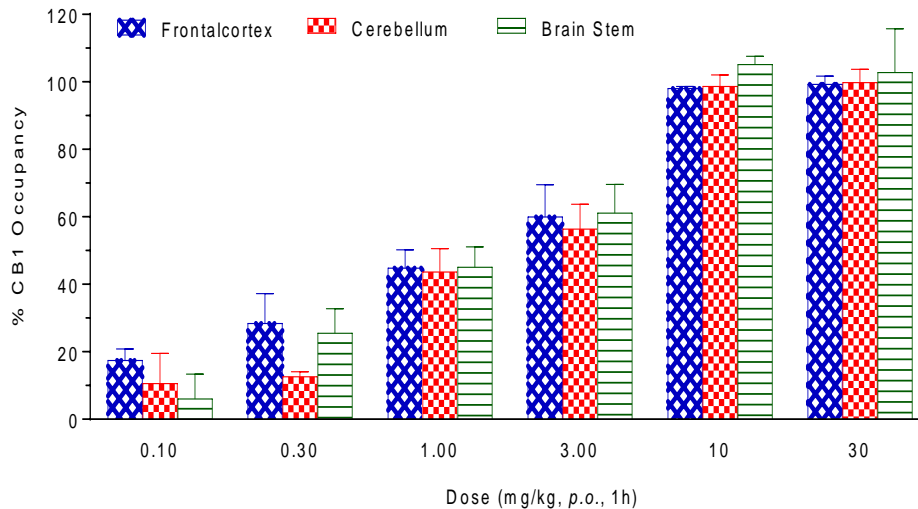


Specific region : Cerebellum/Brain stem/Frontal cortex

Tracer: AM-251

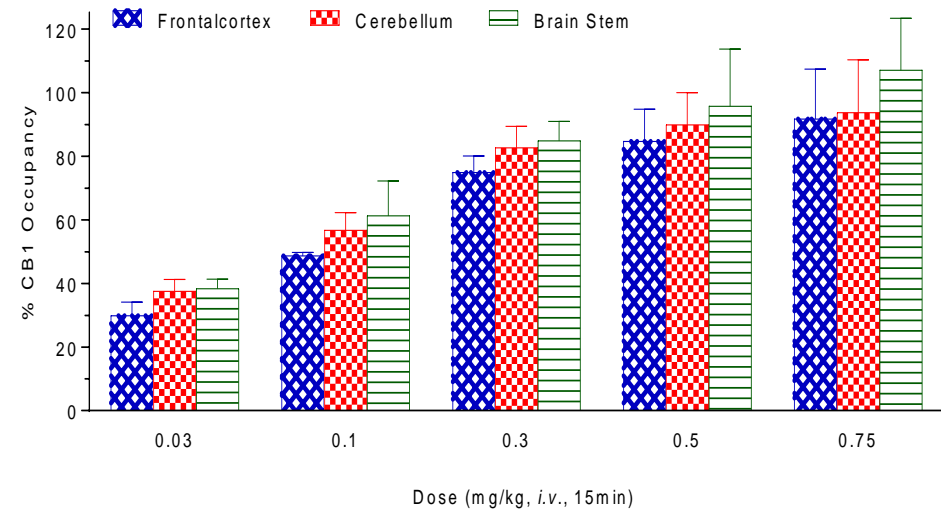
Rimonabant (SR141716A)

ED₅₀ at Suven = 1.09-1.53 mg/kg, *p.o.*



AM281

ED₅₀ at Suven = 0.053-0.089 mg/kg, *i.v.*



LC-MS/MS Based Receptor Occupancy

Adenosine A_{2A} in Rats

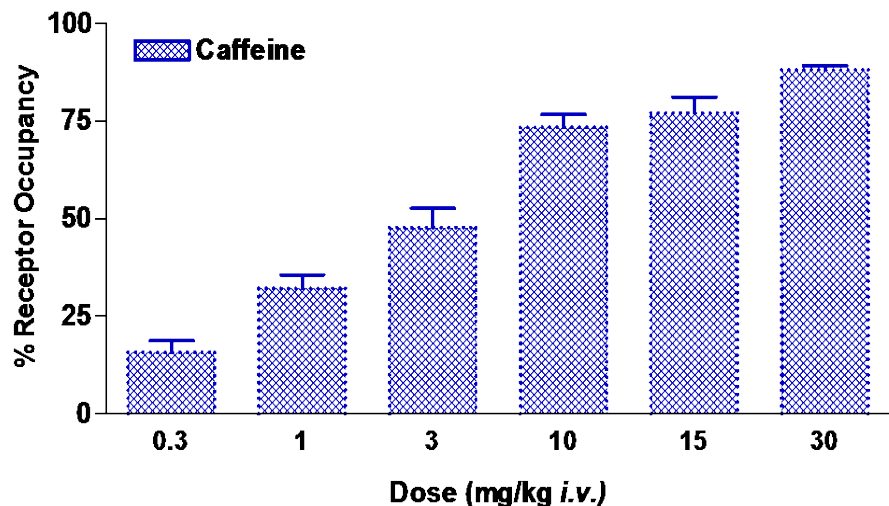


Specific region: Striatum

Non specific region: Cerebellum

Tracer: SCH442416

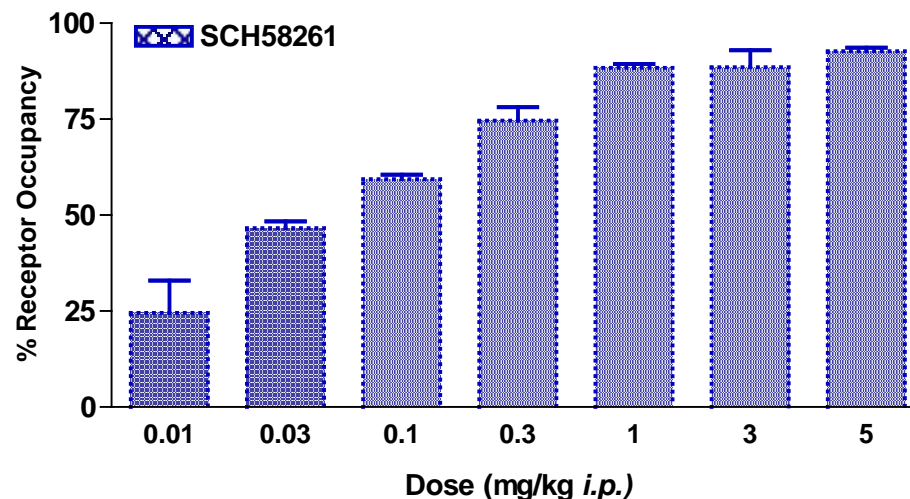
Caffeine



SUVEN ED₅₀ = 3.20 mg/kg, i.v.

Neurology., 2002 and 2003

SCH58261



SUVEN ED₅₀ = 0.05 mg/kg, i.p.

The calculated % receptor occupancy ED₅₀ value from non-radiolabeled tracer is comparable to reported ED₅₀ from study measured using radiolabeled tracer

LC-MS/MS Based Receptor Occupancy

$\alpha 4\beta 2$ nACh in Rats



Specific region : Thalamus

Non specific region: Cerebellum

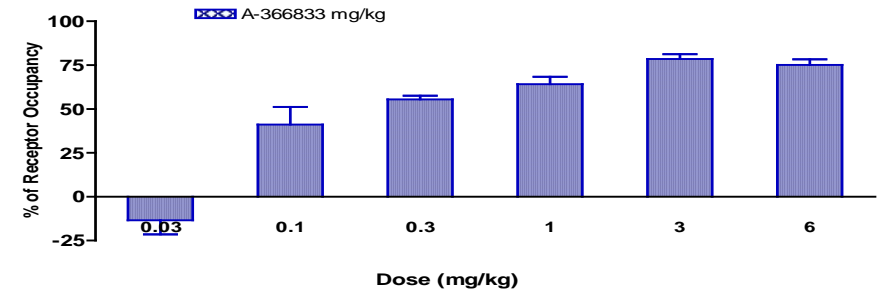
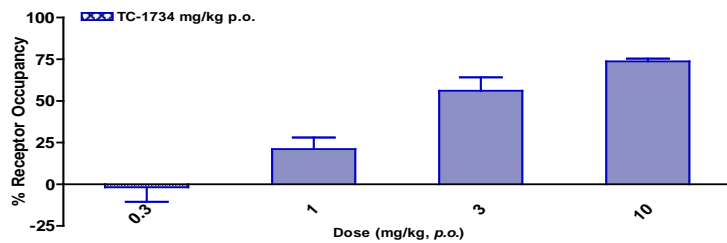
Tracer: ZW-104

TC1734

Ki = 1.5 nM

A366883

Ki = 1.77 nM

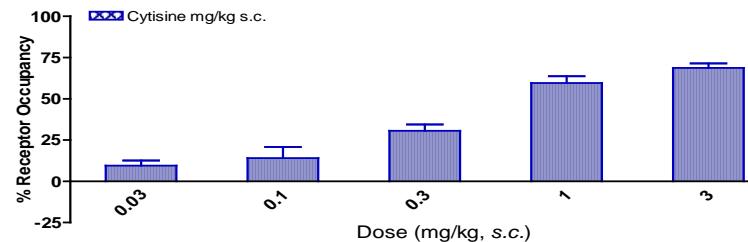


SUVEN ED₅₀ = 1.52 mg/kg, p.o.
Reported ED₅₀ = 0.32 mg/kg, p.o.

SUVEN ED₅₀ = 0.42 mg/kg, i.p.
Reported ED₅₀ = NA

Cytisine

Ki = 0.17 nM



SUVEN ED₅₀ = 0.60 mg/kg, s.c.
Reported ED₅₀ = 0.22 mg/kg, s.c.

SFN-2008.

The calculated % receptor occupancy ED₅₀ value from non-radiolabeled tracer is comparable to reported ED₅₀ from study measured using radiolabeled tracer

LC-MS/MS Based Receptor Occupancy

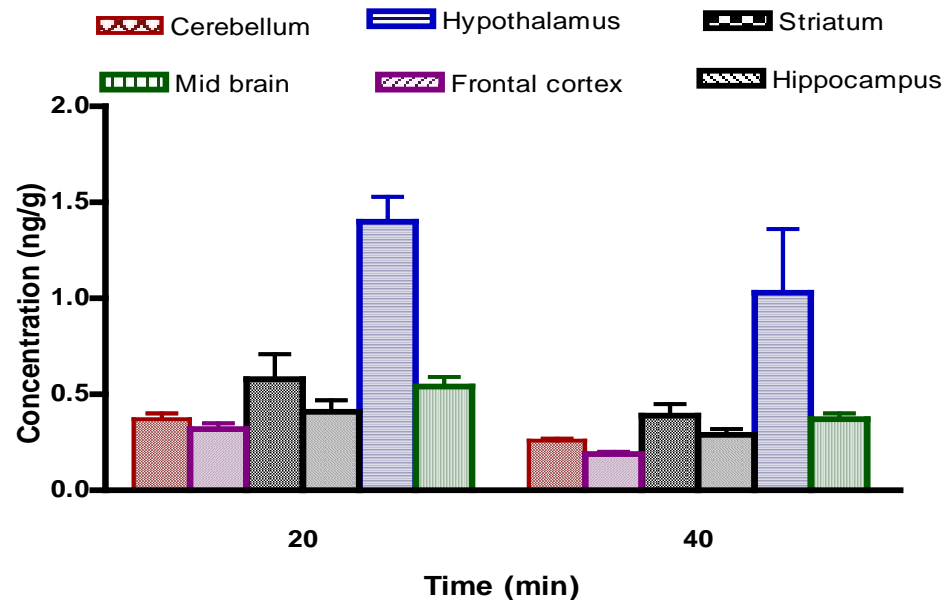
α_7 nACh in Rats



Specific region: Hypothalamus

Non specific region: Cerebellum

Tracer: Methyllylcaconitine (MLA)



MLA, shown high specific binding in hypothalamus and cerebellum as non specific region. This tracer can be employed in screening the ligands specific to α_7 receptor using ratio method

R. Nirogi et al. *Journal Of Pharmacological and Toxicological Methods*, 66 (2012), 22-28



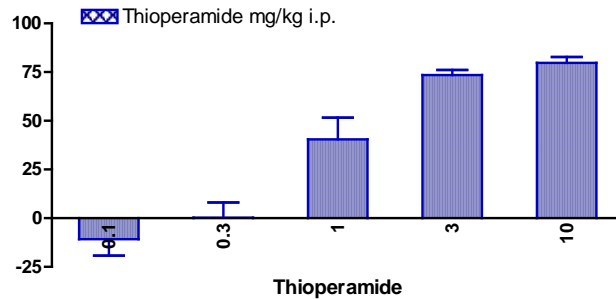
LC-MS/MS Based Receptor Occupancy

Histamine H3 in Rats

Specific region: Frontal Cortex

Thioperamide

Ki = 4 nM



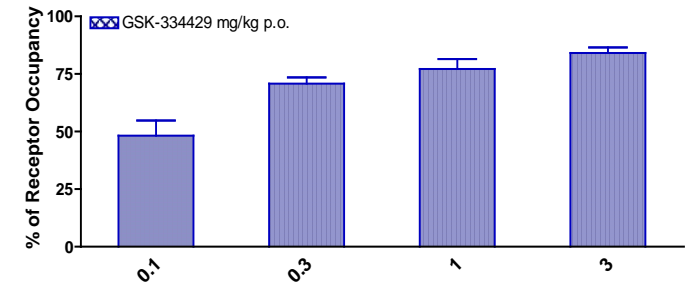
SUVEN ED₅₀ = 1.58 mg/kg, i.p.
Reported ED₅₀ = 2.70 mg/kg, i.p.

Non specific region: Cerebellum

Tracer: GSK-189254

GSK334429

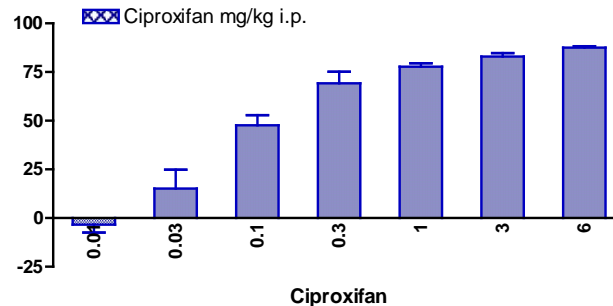
Ki = 5.9 nM



SUVEN ED₅₀ = 0.14 mg/kg, p.o.
Reported ED₅₀ = 0.35 mg/kg p.o.

Ciproxifan

Ki = 0.5 nM



SUVEN ED₅₀ = 0.10 mg/kg, i.p.
Reported ED₅₀ = 0.14 mg/kg, i.p.

The calculated % receptor occupancy ED₅₀ value from non-radiolabeled tracer is comparable to reported ED₅₀ from study measured using radiolabeled tracer

LC-MS/MS Based Receptor Occupancy

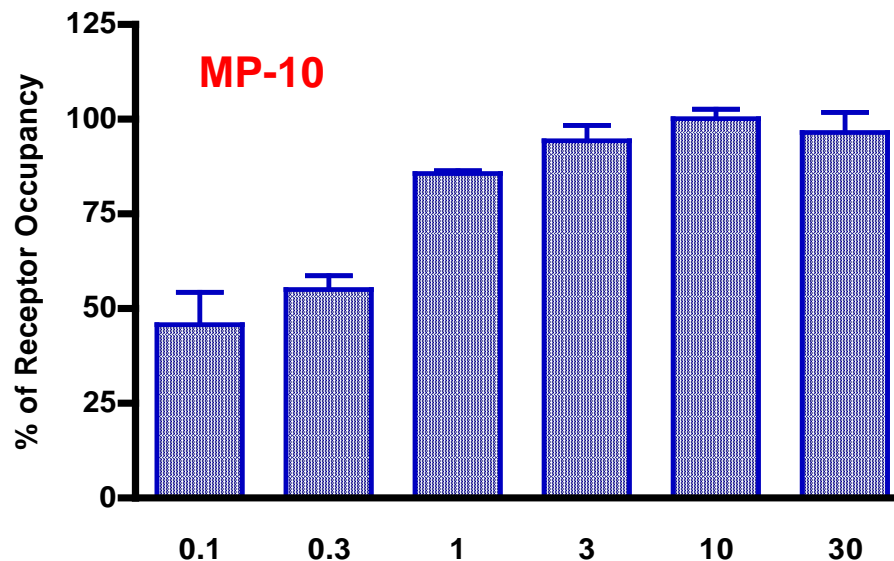
Phosphodiesterase 10 in Rats



Specific region: Striatum

Non specific region: Thalamus

Tracer: AMG 7980



MP-10, mg/kg p.o.

SUVEN ED₅₀ = 0.57 mg/kg, p.o.

Reported ED₅₀ = 2.3 mg/kg, p.o.

Essa *et al.* Journal of Medicinal Chemistry, 2012, (55), 4776-4787

LC-MS/MS Based Receptor Occupancy

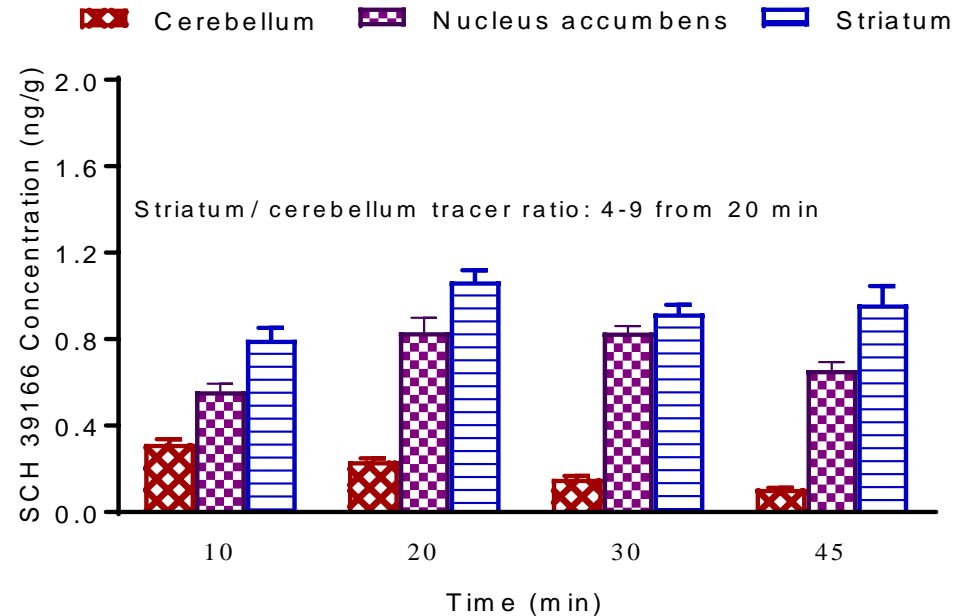
Dopamine D1 in Rats



Specific region: Striatum

Non specific region: Cerebellum

Tracer: SCH 39166



SCH 39166, shown high specific binding in striatum & nucleus accumbens regions and cerebellum as non specific region. This tracer can be employed in screening the ligands specific to D1 receptor using ratio method.



LC-MS/MS Based Receptor Occupancy

Dopamine D2 in Rats

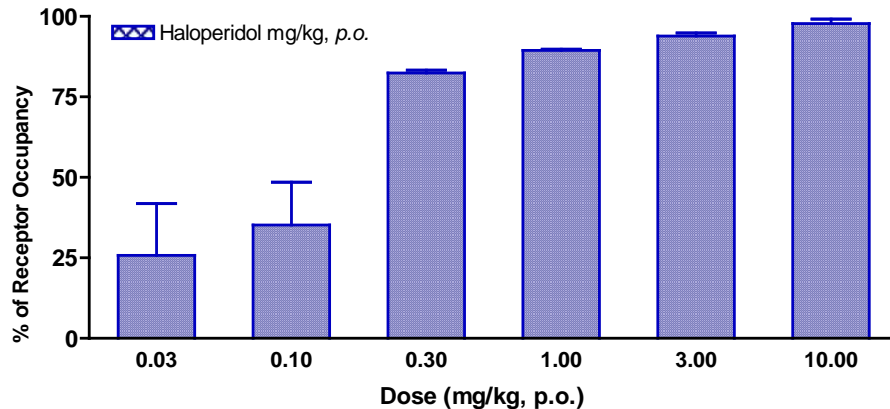
Specific region: Striatum

Non specific region: Cerebellum

Tracer: Raclopride

Haloperidol

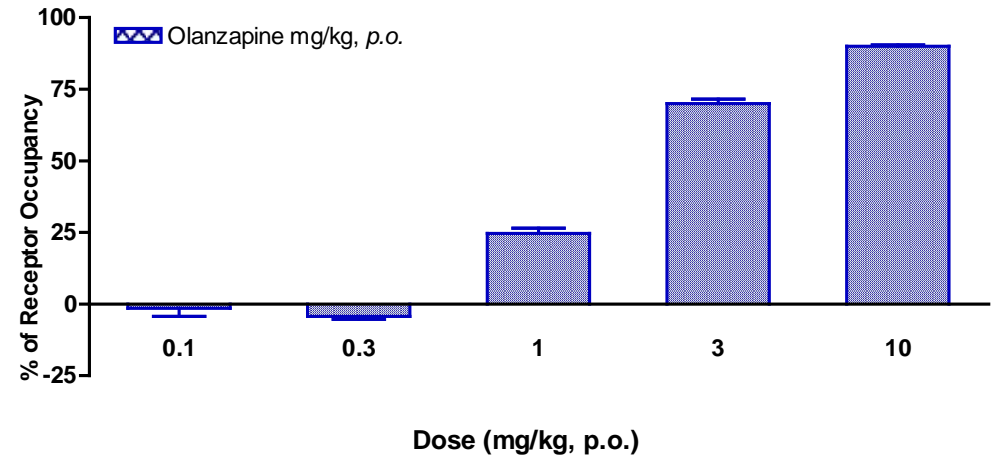
Ki = 1.4 nM



SUVEN ED₅₀ = 0.12 mg/kg, p.o.
Reported ED₅₀ = 0.20 mg/kg, p.o.

Olanzapine

Ki = 22.4 nM



SUVEN ED₅₀ = 1.93 mg/kg, p.o.
Reported ED₅₀ = 2.10 mg/kg, p.o.

Life Sciences., 2005 and 2006.

The calculated % receptor occupancy ED₅₀ value from non-radiolabeled tracer is comparable to reported ED₅₀ from study measured using radiolabeled tracer

LC-MS/MS Based Receptor Occupancy

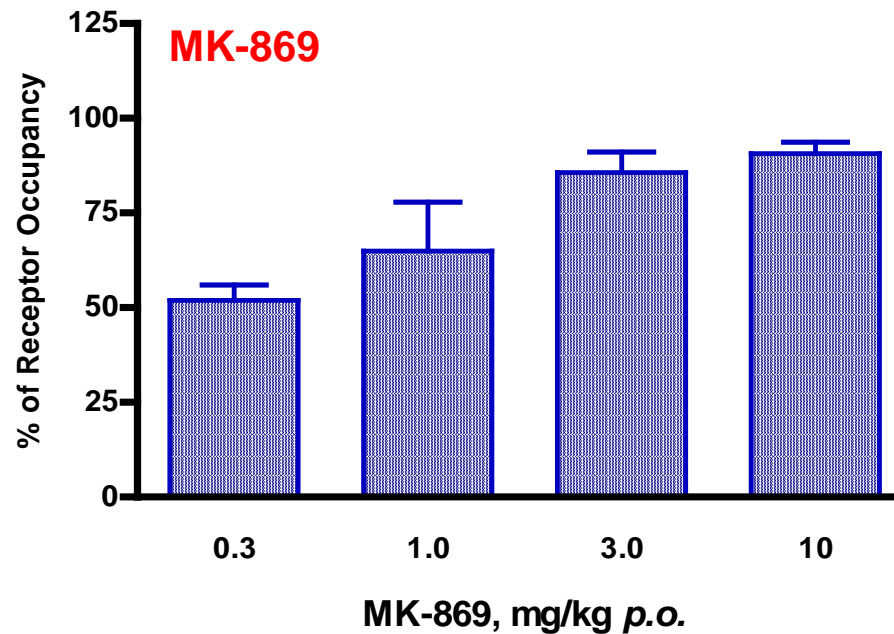
Neurokinin 1 in Rats



Specific region: Striatum / Habenula

Non specific region: Cerebellum

Tracer: GR205171



$ED_{50} = 0.30$ mg/kg, p.o.

LC-MS/MS Based Receptor Occupancy

Adrenergic alpha 1 in Rats

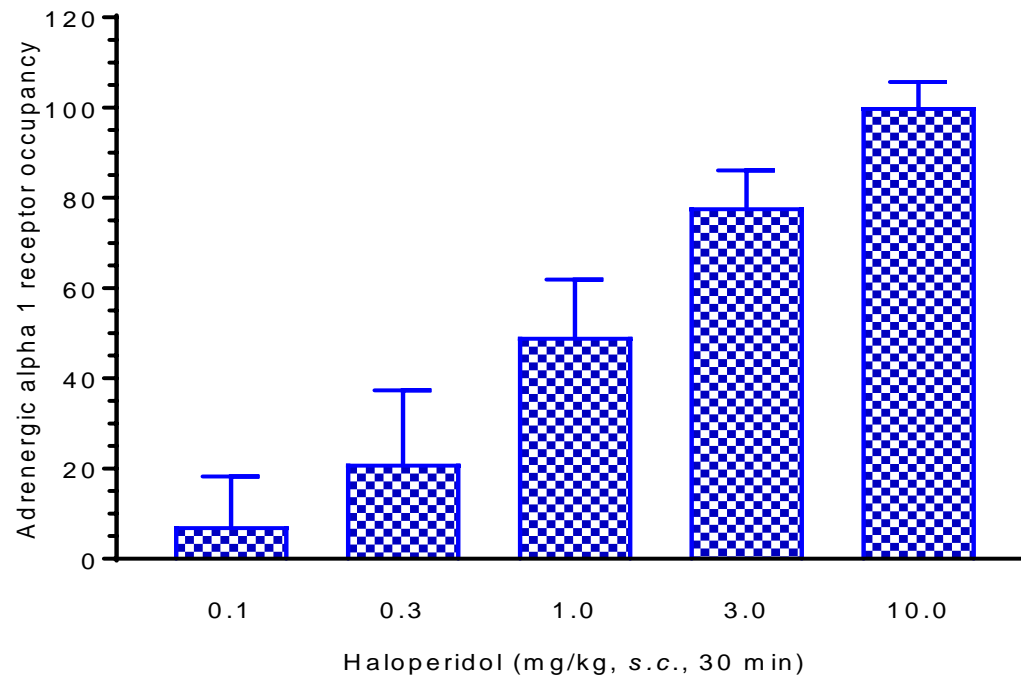


Specific region: Frontal cortex

Non specific region: Cerebellum

Tracer: Prazosin

Haloperidol



ED₅₀ at Suven = 1.05 mg/kg, s.c.

LC-MS/MS Based Receptor Occupancy

mGlu5 in Rats

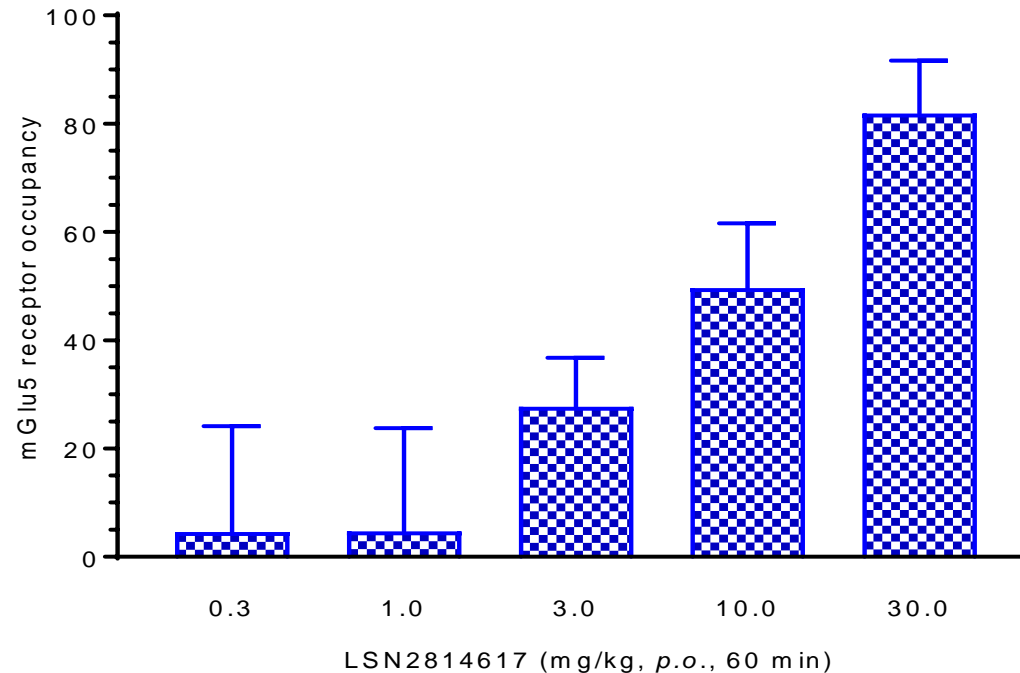


Specific region: Striatum

Non specific region: Cerebellum

Tracer: mPEPy or ABP688

LSN2814617



ED₅₀ at Suven = 8.81 mg/kg, p.o.

LC-MS/MS Based Receptor Occupancy

Sigma-1 in Rats

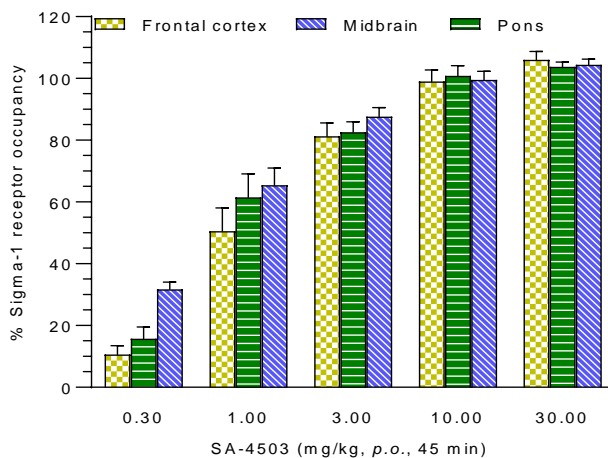


Specific region: Frontal cortex/ midbrain/ pons

Tracer: FTC-146

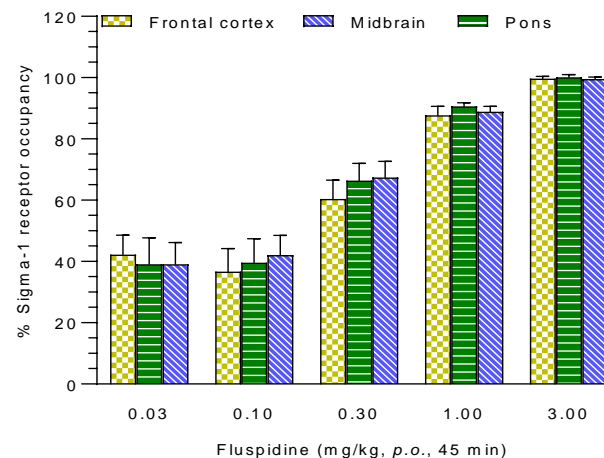
SA-4503

ED₅₀ at Suven
0.58 - 1.04 mg/kg, *p.o*



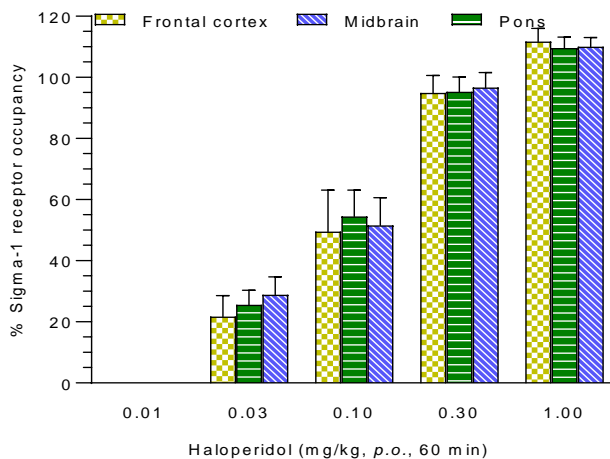
Fluspidine

ED₅₀ at Suven
0.09 - 0.11 mg/kg, *p.o*



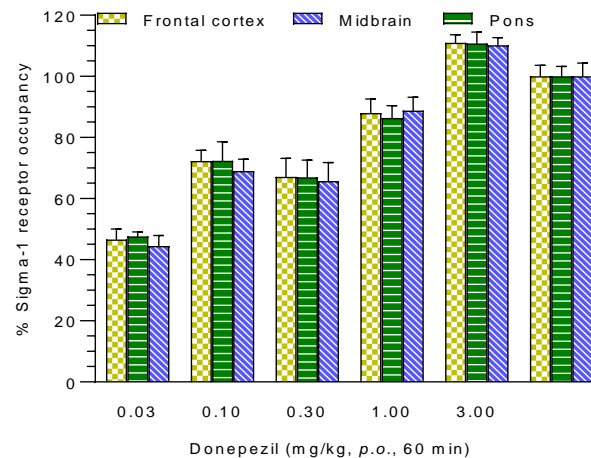
Haloperidol

ED₅₀ at Suven
0.08 - 0.09 mg/kg, *p.o*



Donepezil

ED₅₀ at Suven
0.035 - 0.043 mg/kg, *p.o*



LC-MS/MS Based Receptor Occupancy

Monoamine oxidase (MAO)-A in Rats

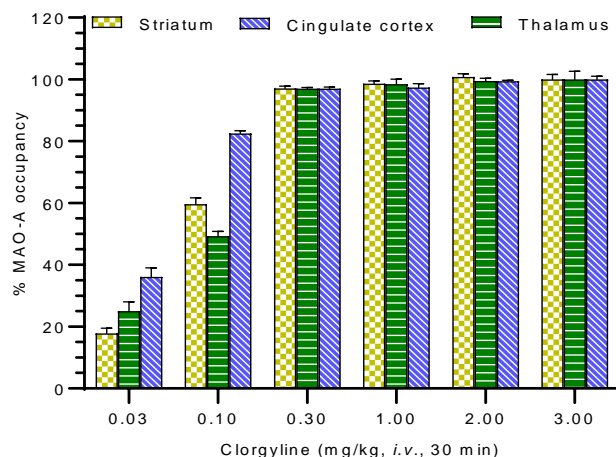


Specific region: Striatum/ cingulate cortex/ thalamus

Tracer: Harmine

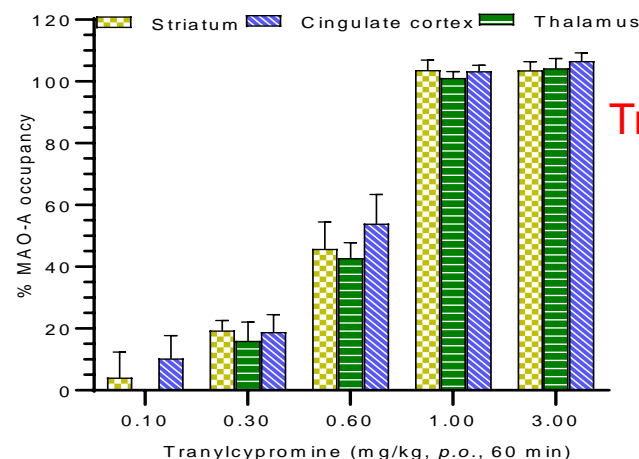
Clorgyline

ED₅₀ at Suven
0.041 – 0.081 mg/kg, *i.v.*



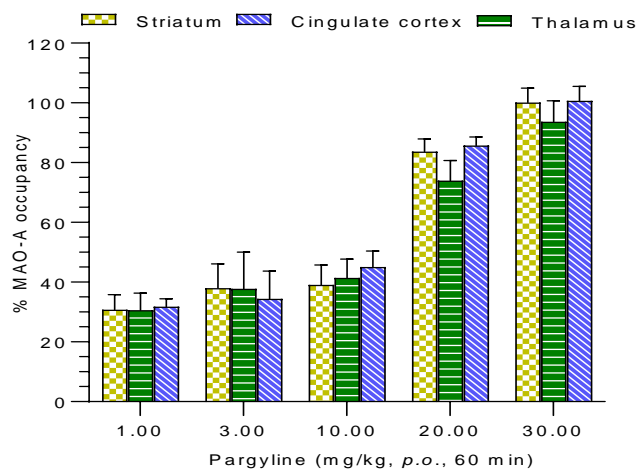
Tranlycypromine

ED₅₀ at Suven
0.525 – 0.620 mg/kg, *p.o.*



Pargyline

ED₅₀ at Suven
5.0 – 5.6 mg/kg, *p.o.*



LC-MS/MS Based Receptor Occupancy

Monoamine oxidase (MAO)-B in Rats

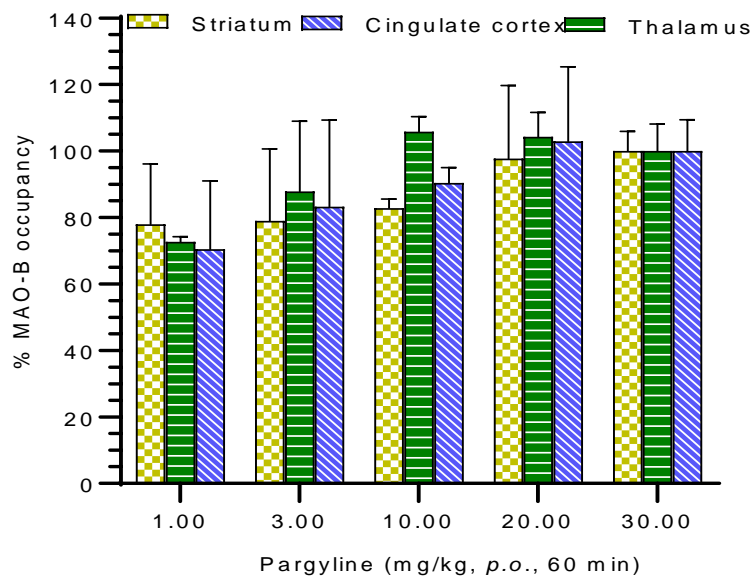


Specific region: Striatum/ cingulate cortex/ thalamus

Tracer: R (-) Deprenyl

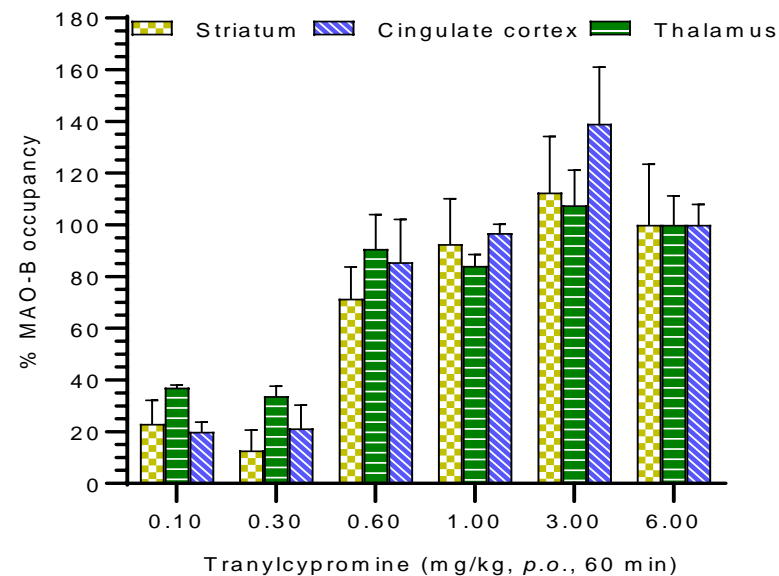
Pargyline

ED₅₀ at Suven = 0.11 – 0.51 mg/kg, *p.o*



Tranlycypromine

ED₅₀ at Suven = 0.25 – 0.48 mg/kg, *p.o*



Receptor occupancy using [3H] tracers

Overview



- We have added **Tricarb 3110TR*** to our fleet of high-end instruments.
- This State- of- the- Art Technology Adds to Our Capability in Measurement of Pre-clinical Receptor Occupancy for Validated and Novel Targets
- Suven has Capability to Measure RO by Labeled and Non- Labeled Tracers



Filtration of tissue homogenate enhance the specific binding for low density targets

*Tricarb 3110TR is a vial-based bench-top liquid scintillation analyzer (Make- PerkinElmer) and the most versatile and sensitive instrument available for detecting small amounts of alpha, beta and gamma radioactivity.

Receptor occupancy using [3H] tracers

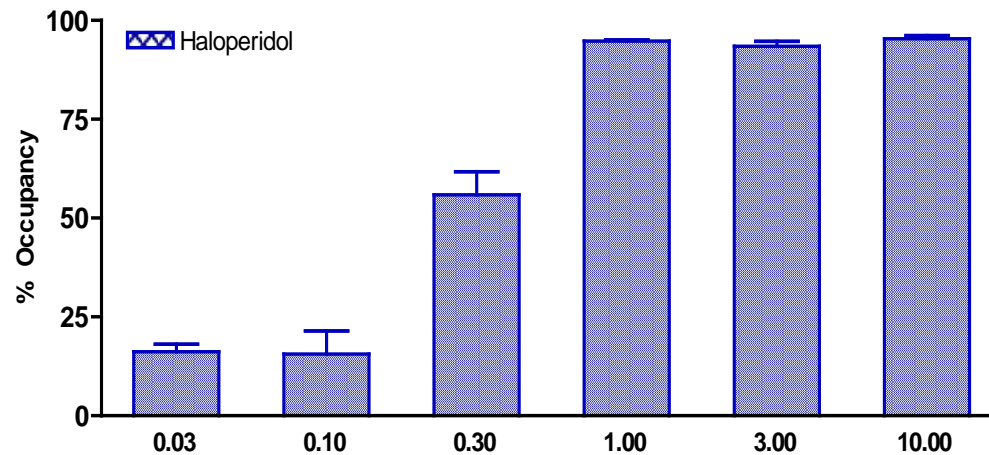
D2 Receptor Occupancy Using [3H] Raclopride as a Tracer



Specific region: Striatum

Non specific region: Cerebellum

Tracer: ³H-Raclopride



RO ED₅₀ = 0.25 mg/kg, p.o.

Reported RO ED₅₀ = 0.3 ± 1.1 mg/kg, p.o.

Contacts



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