In Vivo Receptor Occupancy Capabilities

Discovery Research
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Contents

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 α7 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

Formulation

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

Animal model: rat/ mouse/ guinea pig

Receptor occupancy and exposure correlation

LC-MS/MS based

- 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

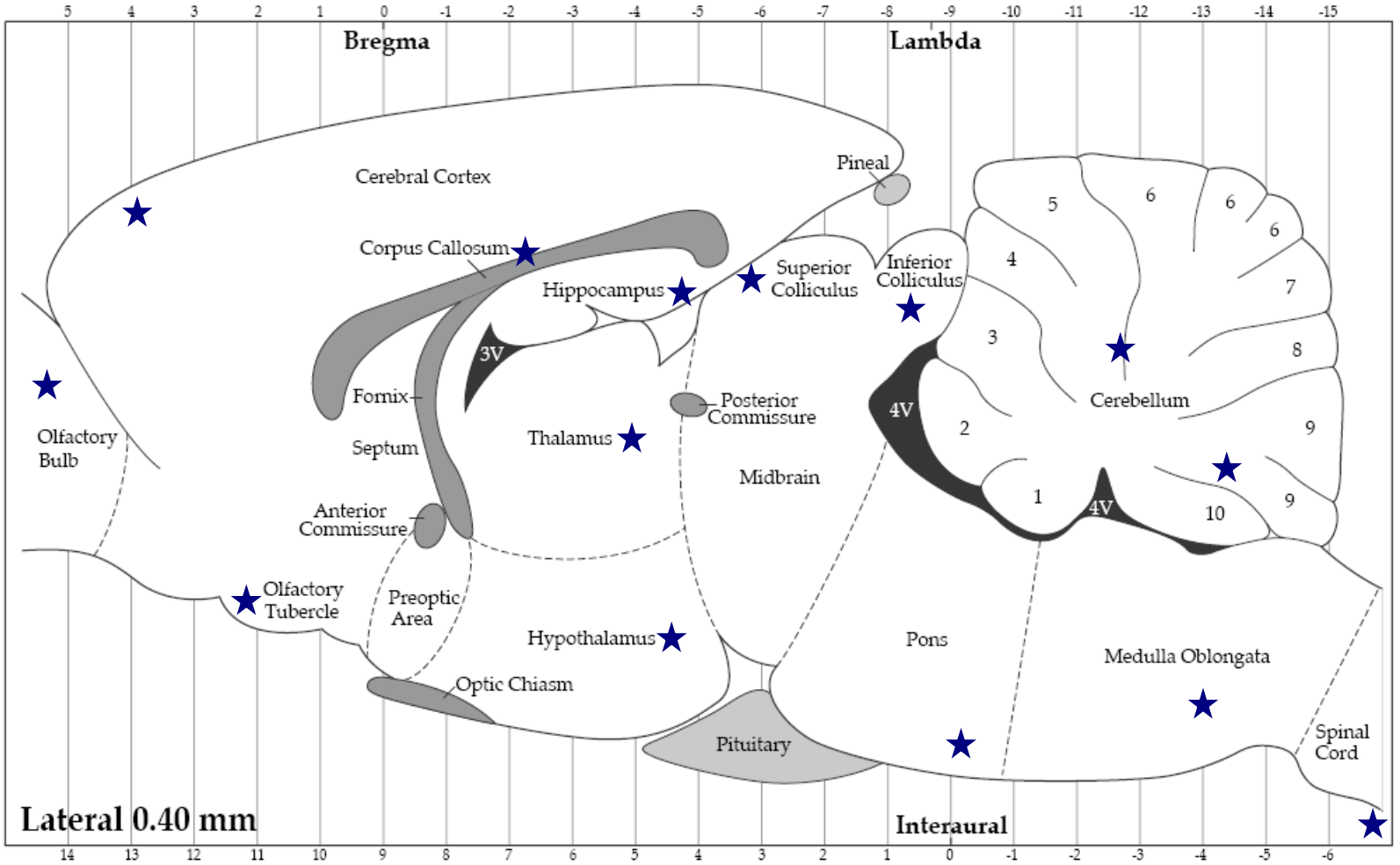
Liquid scintillation analyzer (LSA) based

- Radiolabelled ([^{3}H]) 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 Suven

LC-MS/MS Based Receptor Occupancy

Overview: Validated Targets

Receptor	Tracer
#, * 5-HT_{1A}	WAY-100635■
5-HT_{1B}	AZ10419369
* 5-HT_{2A}	MDL-100907
5-HT_{2C}	SB242084
5-HT_4	SB207145
5-HT_6	Lu AE60157■
NET	S,S-MeNER
DAT	WIN 35428
#SERT	DASB
$GABA_A$	Flumazenil
CB_1	AM251

Receptor	Tracer
A_{2A}	SCH442416
nAChR $\alpha_4\beta_2$	ZW-104
nAChR α_7	Methyllycaconitine
Histamine H_3	GSK-189254
PDE10	AMG 7980
D_1	SCH39166
* D_2	Raclopride■
NK1	GR205171
mGluR5	mPEPy
Adrenergic α1A	Prazosin

Receptor	Tracer
Sigma 1	FTC-146
MAO-A	Harmine
MAO-B	Selegiline

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: Adinosine; nAChR: nicotinic acetylcholine receptor; PDE: Phosphodiesterase; D: Dopamine; NK: Neurokinin; mGluR: metabotropic glutamate receptor, MAO: monoamine oxidase

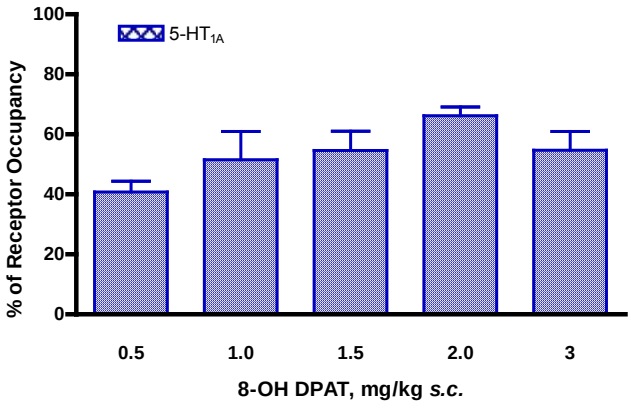
LC-MS/MS Based Receptor Occupancy

5-HT_{1A} in Rats

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

8-OHDPAT

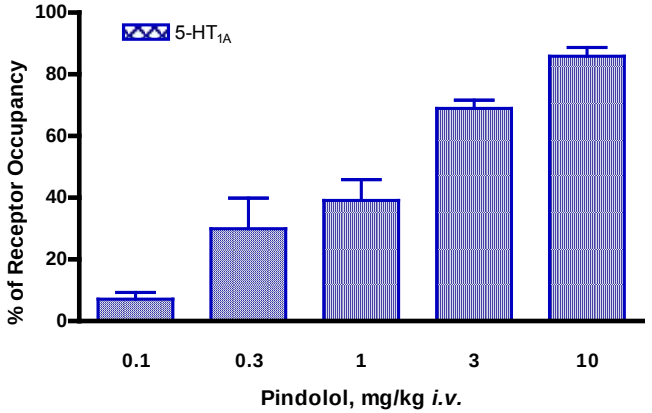
Ki = 0.58 nM



SUVEN ED_{50} = 0.95 mg/kg, s.c.
Reported ED_{50} = 0.60 mg/kg, s.c.

Pindolol

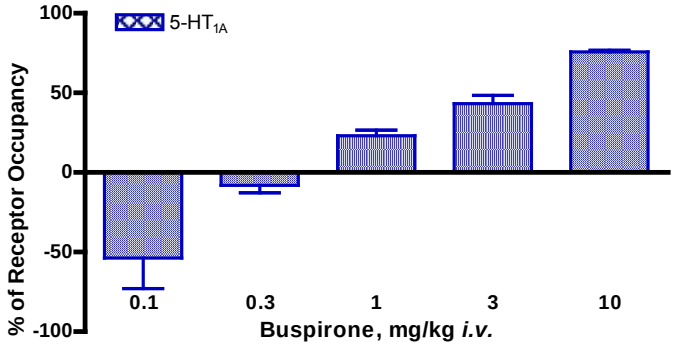
Ki = 14.4 nM



SUVEN ED_{50} = 1.27 mg/kg, i.v.
Reported ED_{50} = 0.44 mg/kg, i.v.

Buspirone

Ki = 8.9 nM



SUVEN ED_{50} = 3.74 mg/kg, i.v.
Reported ED_{50} = 5.00 mg/kg, i.v.

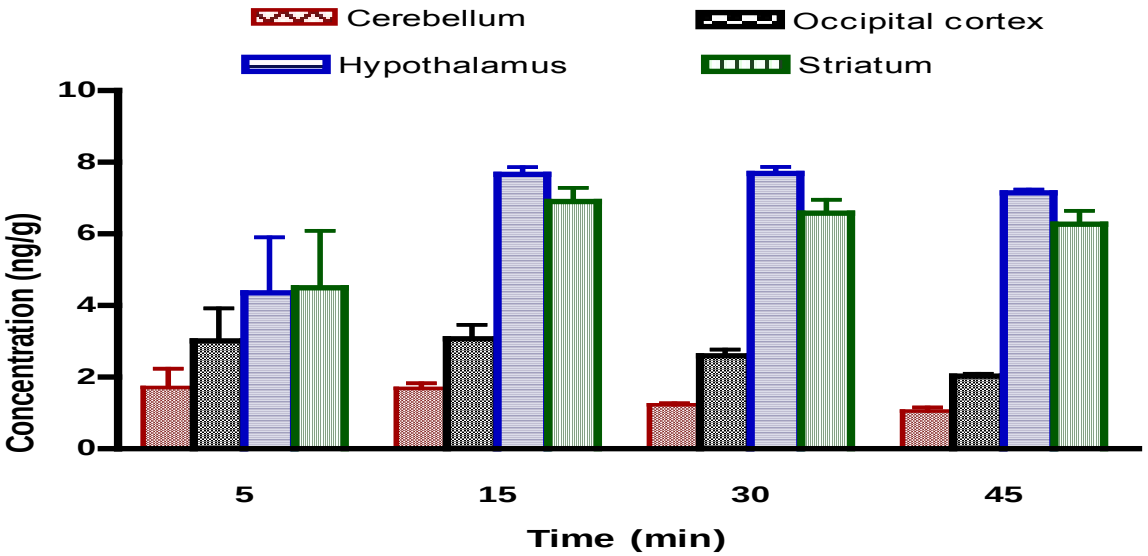
Synapse., 2000; Psychopharmacology., 1997; Neuropsychopharmacology., 2004; Nuclear Medicine & Biology., 2000.

The calculated % receptor occupancy ED_{50} value from non-radiolabeled tracer is comparable to reported ED_{50} 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

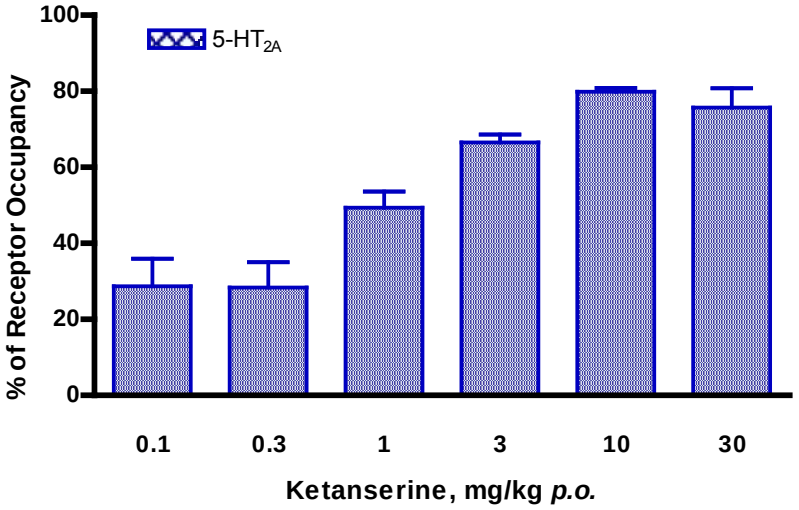
Specific region: Frontal cortex

Non specific region: Cerebellum

Tracer: MDL 100907

Ketanserin

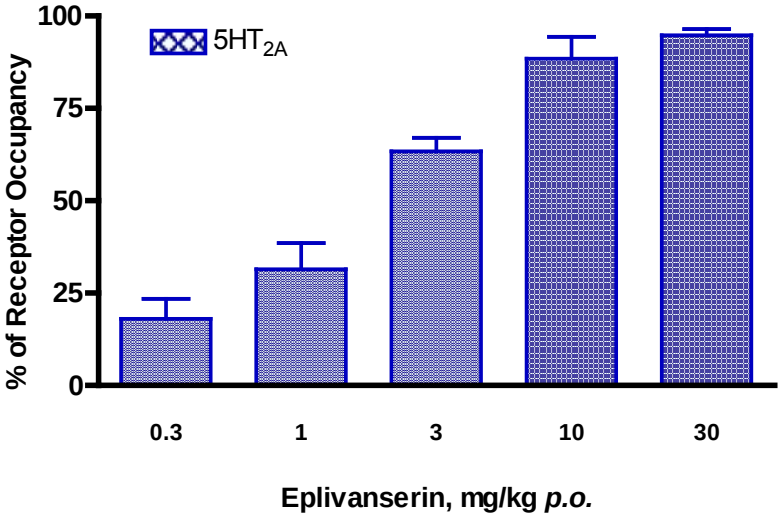
Ki = 3 nM



SUVEN ED_{50} = 1.04 mg/kg, p.o.
Reported ED_{50} = 0.32 mg/kg, p.o.

Eplivanserin

Ki = 0.12 nM



SUVEN ED_{50} = 1.79 mg/kg, p.o.
Reported ED_{50} = 1.50 mg/kg, p.o.

Life Science., 2007.

The calculated % receptor occupancy ED_{50} value from non-radiolabeled tracer is comparable to reported ED_{50} from study measured using radiolabeled tracer

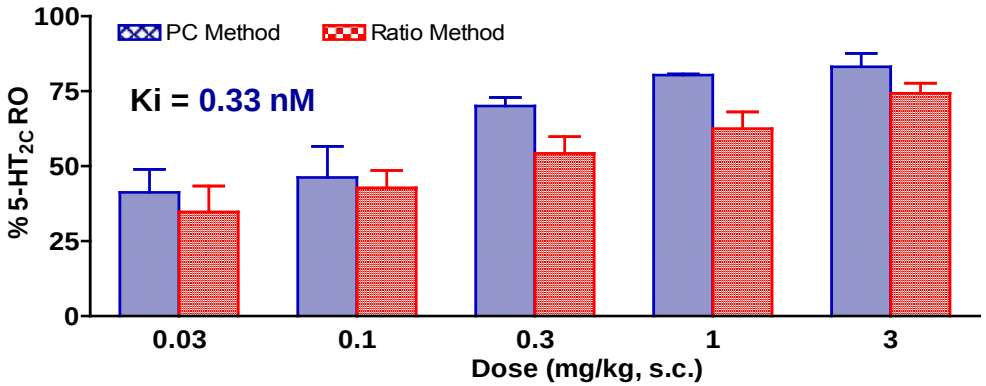
LC-MS/MS Based Receptor Occupancy

5-HT_{2C} in Rats

Specific region: Choroid plexus Non specific region: Cerebellum Tracer: SB 242084

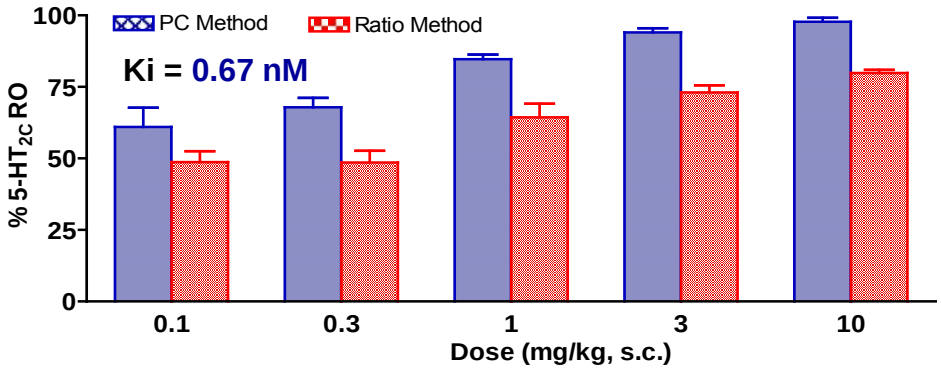
Ritanserin

SUVEN ED_{50} = 0. 08 mg/kg, *s.c.*



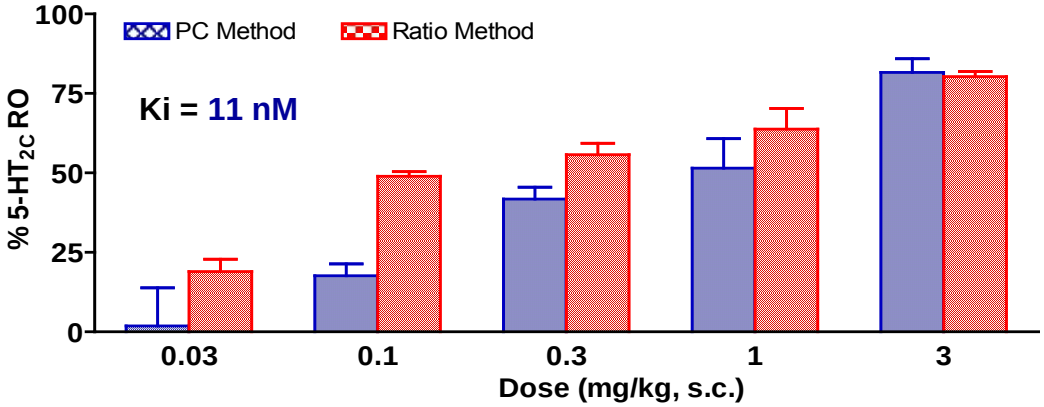
Mianserin

SUVEN ED_{50} = 0.04 mg/kg, *s.c.*



Olanzapine

SUVEN ED_{50} = 0.68 mg/kg, *s.c.*



LC-MS/MS Based Receptor Occupancy

5-HT_4 in Rats

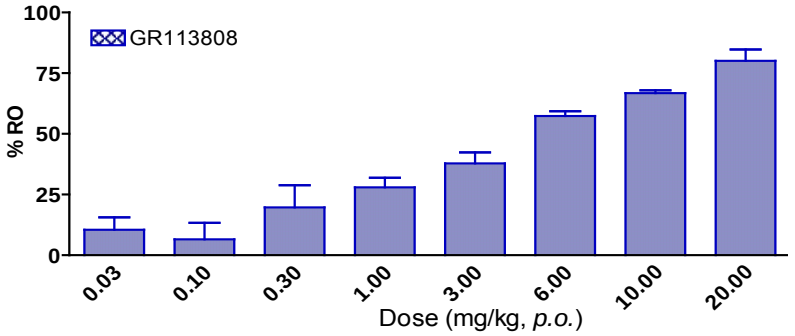
Specific region: Striatum

Non specific region: Cerebellum

Tracer: SB-207145

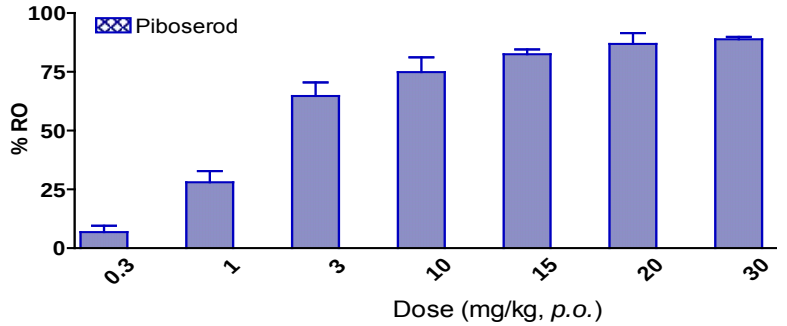
GR113808 (Antagonist)

Ki = 0.3 nM



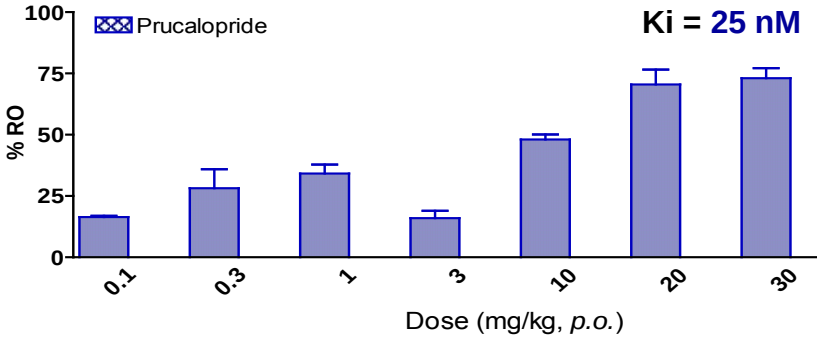
Piboserod (Antagonist)

Ki = 0.12 nM



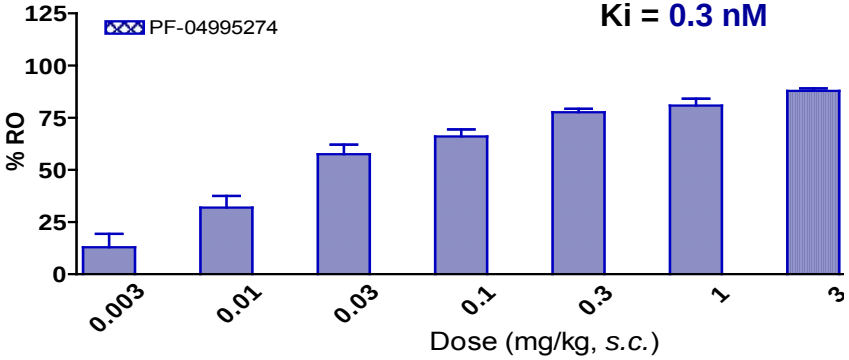
Prucalopride (Agonist)

Ki = 25 nM



PF04995274 (Partial agonist)

Ki = 0.3 nM



Reported ID_{50} = 0.008 mg/kg, s.c.

Obtained ED_{50} = 0.017 mg/kg, s.c.

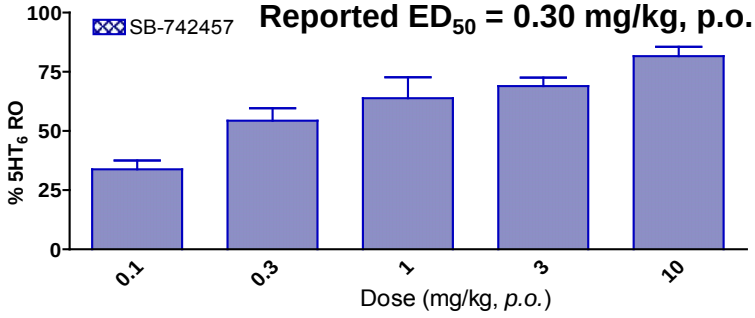
Grimwood et al., Pfizer – Poster No: 11818 - Translational receptor occupancy for the 5-HT4 partial agonist PF-04995274 in rats, non-human primates and healthy volunteers – ICAD-2011 - Paris

LC-MS/MS Based Receptor Occupancy

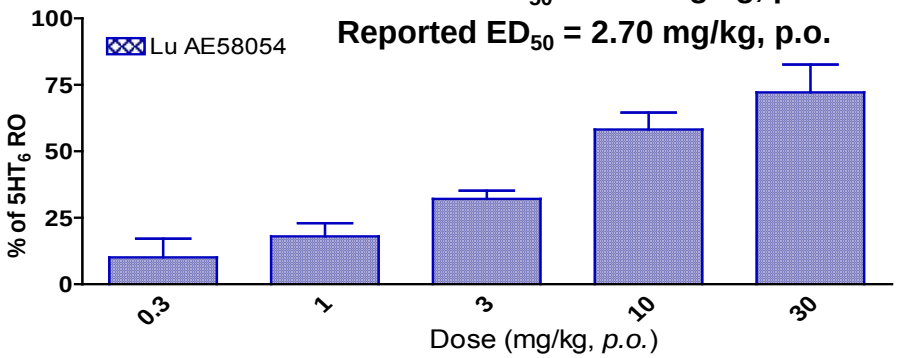
5-HT_6 in Rats

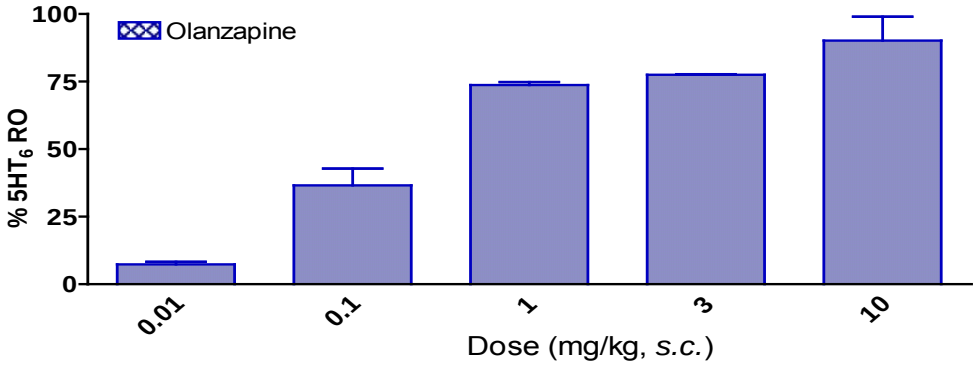
Specific region: Striatum | Non specific region: Cerebellum | Tracer: LuAE-60157

Obtained ED_{50} = 0.29 mg/kg, p.o.
Reported ED_{50} = 0.30 mg/kg, p.o.



Obtained ED_{50} = 5.16 mg/kg, p.o.
Reported ED_{50} = 2.70 mg/kg, p.o.





Obtained ED_{50} = 0.01 mg/kg, s.c.
Reported ED_{50} = 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

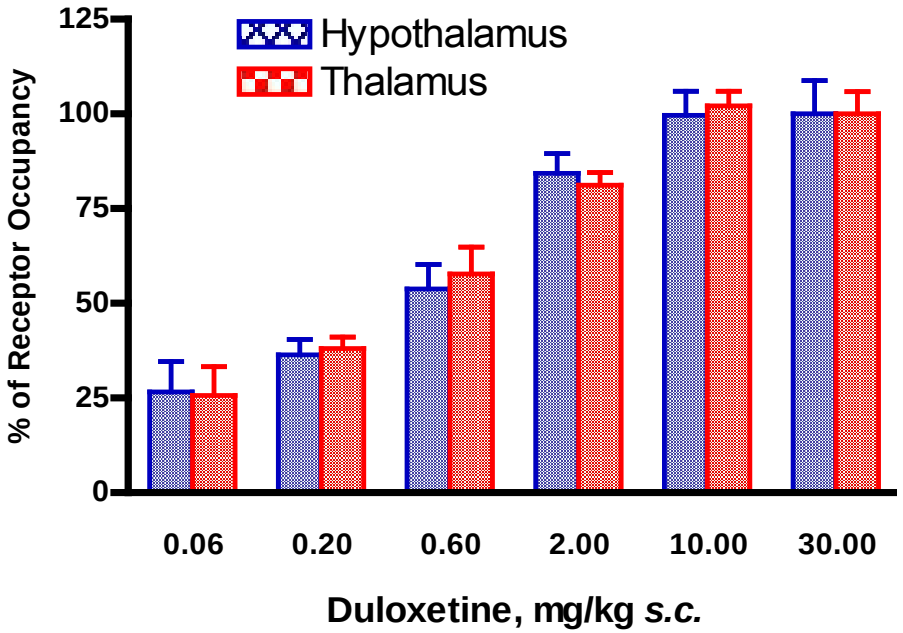
NET in Rats

Specific region: Thalamus/Hypothalamus

Tracer: S,S-MeNER

Duloxetine

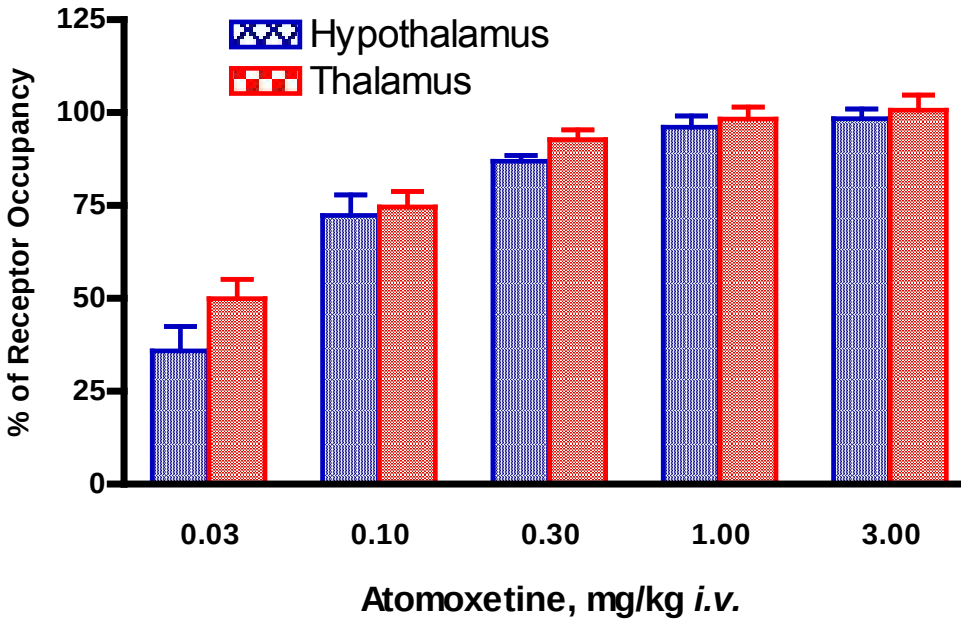
Ki = 10 nM



SUVEN ED_{50} = 0.40 mg/kg, s.c.
Reported ED_{50} = 1.00 mg/kg, s.c.

Atomoxetine

Ki = 5 nM



SUVEN ED_{50} = 0.04 mg/kg, i.v.
Reported ED_{50} = 0.028 mg/kg/h or 0.13 mg/kg, i.v.

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

The calculated % receptor occupancy ED_{50} value from non-radiolabeled tracer is comparable to reported ED_{50} from study measured using radiolabeled tracer

LC-MS/MS Based Receptor Occupancy

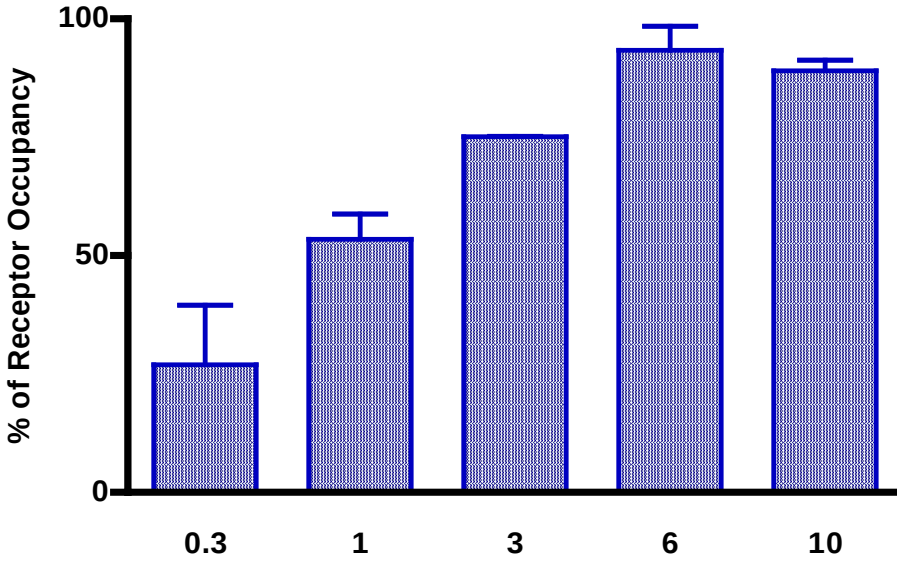
DAT in Rats

Specific region: Striatum

Non specific region: Cerebellum

Tracer: WIN 35428

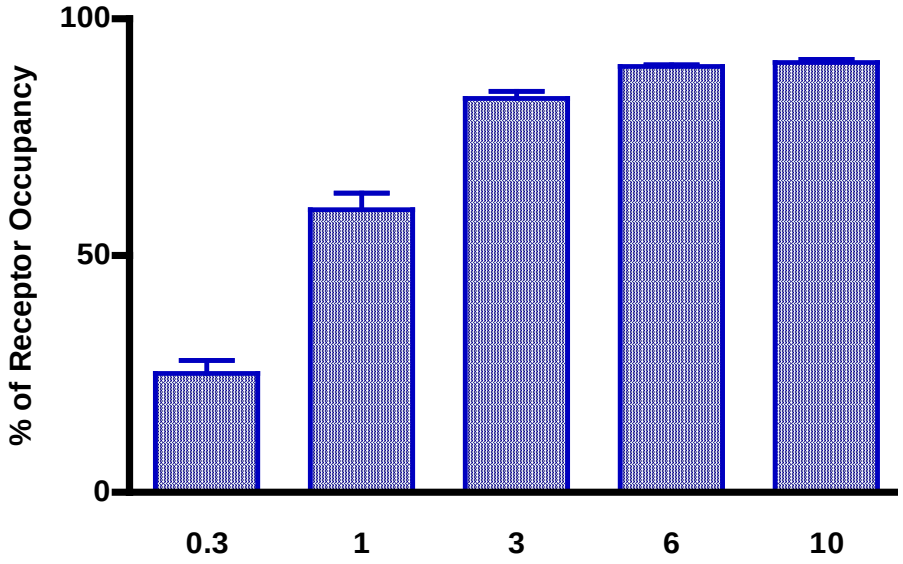
GR12909



SUVEN ED_{50} = 0.89 mg/kg, i.v.

Reported ED_{50} = 1.49 mg/kg, i.v.

Nomifensine



SUVEN ED_{50} = 0.65 mg/kg, i.v.

Reported ED_{50} = 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

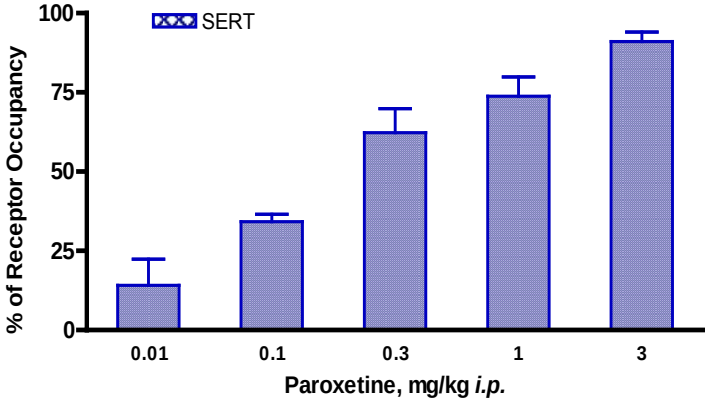
SERT in Rats

Specific region: Frontal cortex

Tracer: DASB

Paroxetine

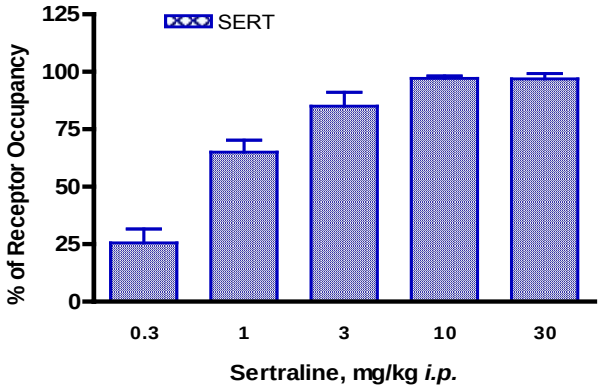
Ki = 0.16 nM



SUVEN ED_{50} = 1.27 mg/kg, i.p.
Reported ED_{50} = 1.00 mg/kg, i.p.

Sertraline

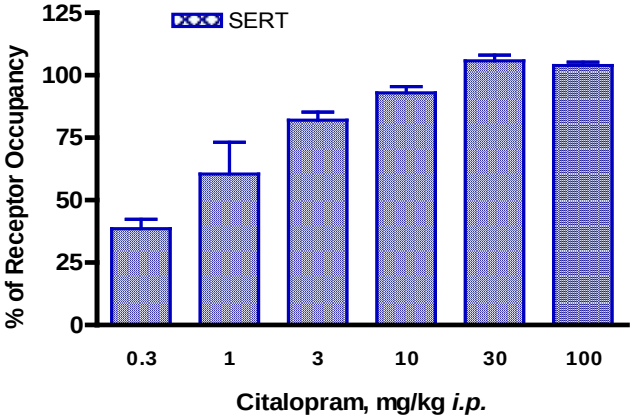
Ki = 0.7 nM



SUVEN ED_{50} = 1.97 mg/kg, i.p.
Reported ED_{50} = 2.60 mg/kg, i.p.

Citalopram

Ki = 0.8 nM



SUVEN ED_{50} = 2.48 mg/kg, i.p.
Reported ED_{50} = 4.70 mg/kg, i.p.

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

The calculated % receptor occupancy ED_{50} value from non-radiolabeled tracer is comparable to reported ED_{50} from study measured using radiolabeled tracer

LC-MS/MS Based Receptor Occupancy

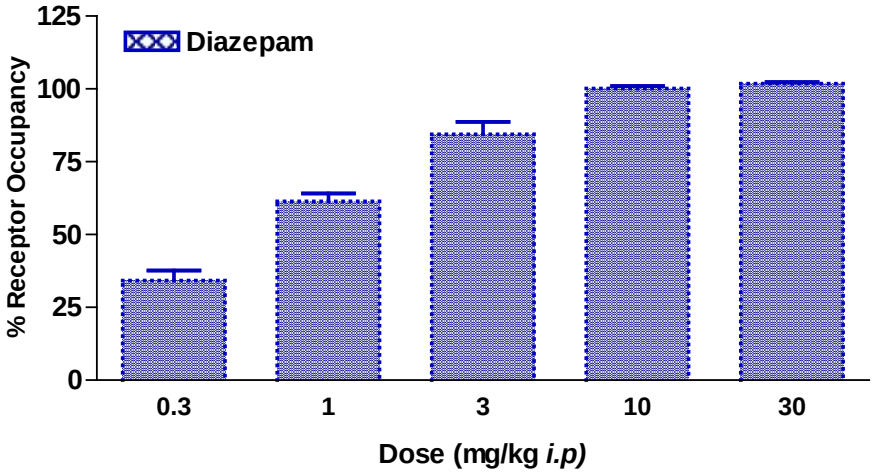
Benzodiazepine $GABA_A$ in Rats

Specific region : Frontal cortex

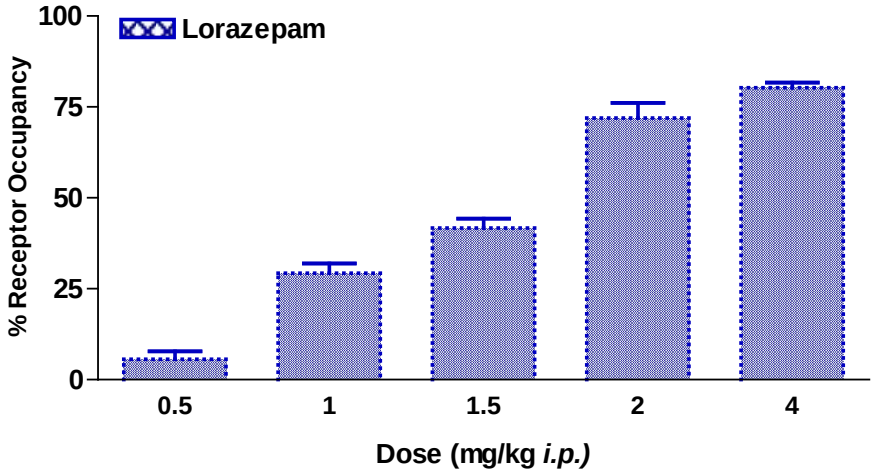
Non specific region: Pons

Tracer: Flumazenil

Diazepam



Lorazepam



JPET., 2007

The calculated % receptor occupancy ED_{50} value from non-radiolabeled tracer is comparable to reported ED_{50} from study measured using radiolabeled tracer.

LC-MS/MS Based Receptor Occupancy

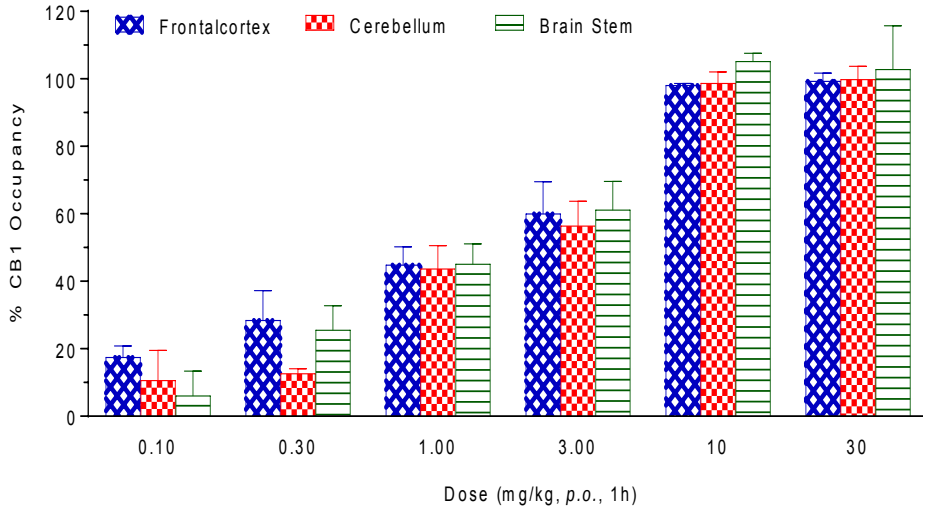
Cannabinoid-1 in Rats

Specific region : Cerebellum/Brain stem/Frontal cortex

Tracer: AM-251

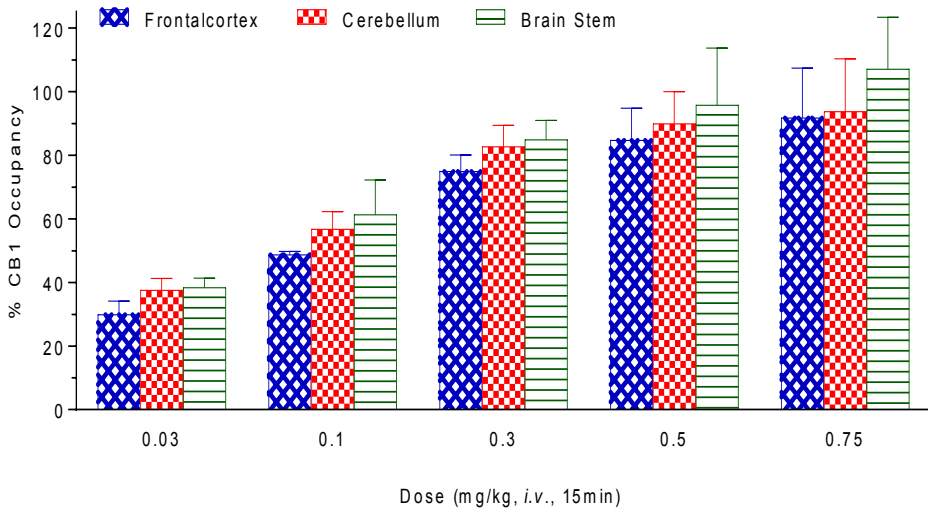
Rimonabant (SR141716A)

ED_{50} at Suven = 1.09-1.53 mg/kg, *p*.o.



AM281

ED_{50} at Suven = 0.053-0.089 mg/kg, *i.v.*



LC-MS/MS Based Receptor Occupancy

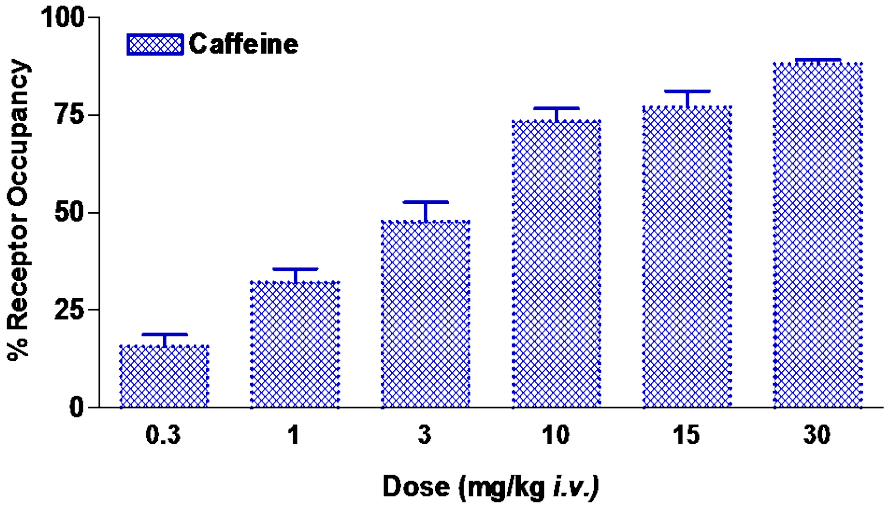
Adenosine A_{2A} in Rats

Specific region: Striatum

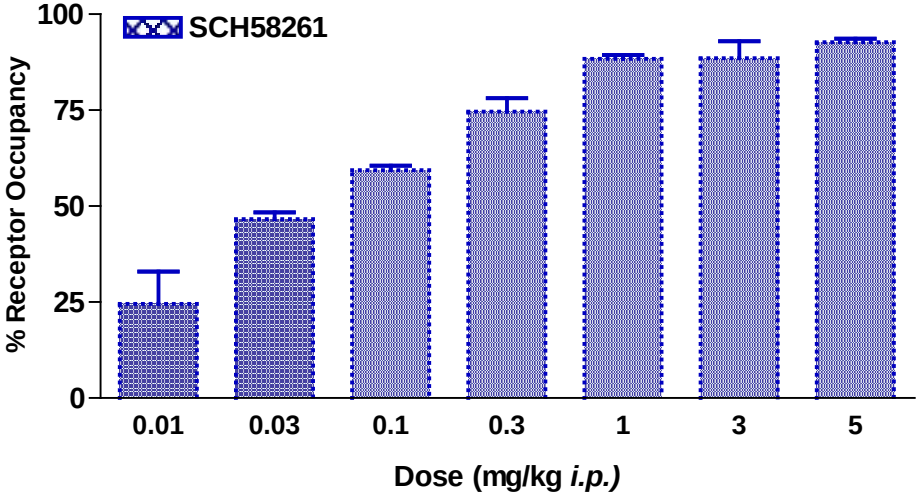
Non specific region: Cerebellum

Tracer: SCH442416

Caffeine



SCH58261



SUVEN ED_{50} = 3.20 mg/kg, i.v.

SUVEN ED_{50} = 0.05 mg/kg, i.p.

Neurology., 2002 and 2003

The calculated % receptor occupancy ED_{50} value from non-radiolabeled tracer is comparable to reported ED_{50} from study measured using radiolabeled tracer

LC-MS/MS Based Receptor Occupancy

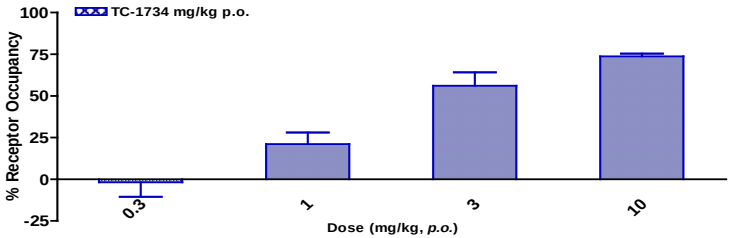
α4β2 nACh in Rats

Specific region : Thalamus

Non specific region: Cerebellum

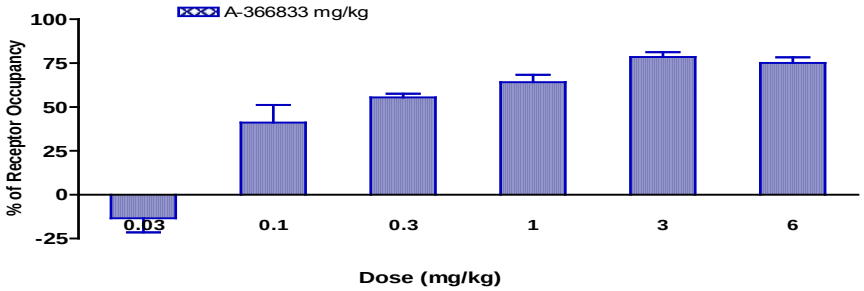
Tracer: ZW-104

TC1734 **Ki = 1.5 nM**



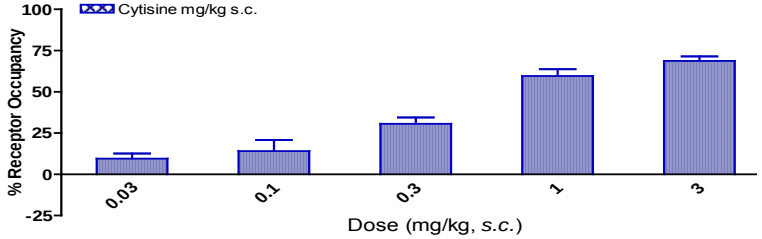
SUVEN ED_{50} = 1.52 mg/kg, p.o.
Reported ED_{50} = 0.32 mg/kg, p.o.

A366883 **Ki = 1.77 nM**



SUVEN ED_{50} = 0.42 mg/kg, i.p.
Reported ED_{50} = NA

Cytisine **Ki = 0.17 nM**



SUVEN ED_{50} = 0.60 mg/kg, s.c.
Reported ED_{50} = 0.22 mg/kg, s.c.

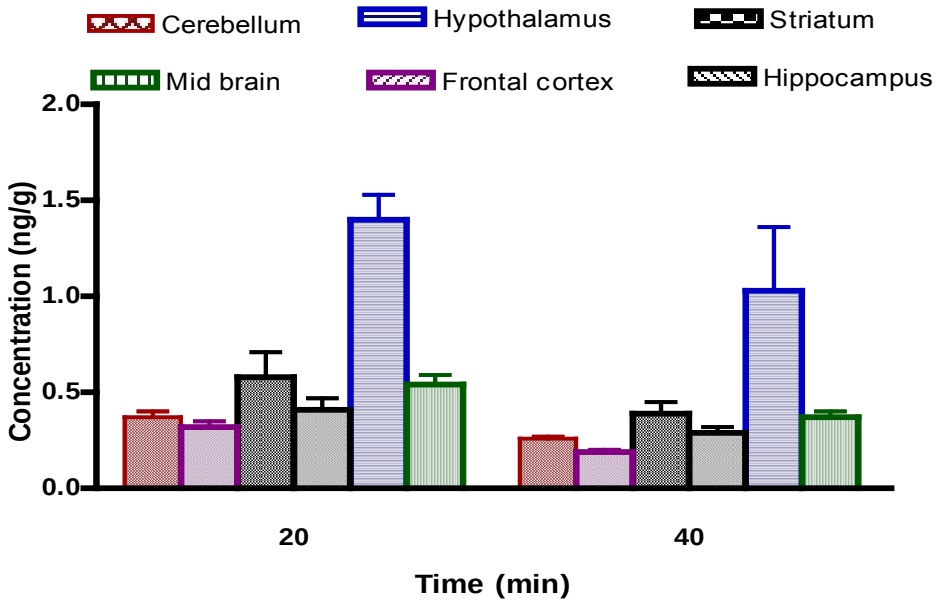
SFN-2008.

The calculated % receptor occupancy ED_{50} value from non-radiolabeled tracer is comparable to reported ED_{50} from study measured using radiolabeled tracer

LC-MS/MS Based Receptor Occupancy

α7 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

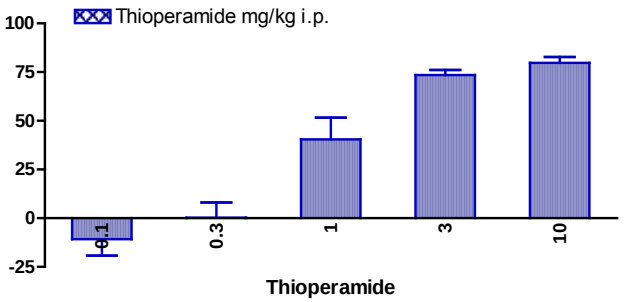
LC-MS/MS Based Receptor Occupancy

Histamine H3 in Rats

Specific region: Frontal Cortex Non specific region: Cerebellum Tracer: GSK-189254

Thioperamide

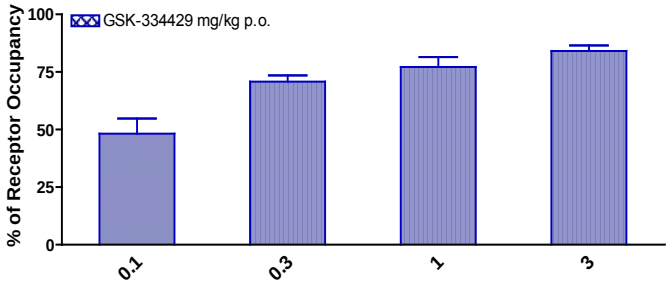
Ki = 4 nM



SUVEN ED_{50} = 1.58 mg/kg, i.p.
Reported ED_{50} = 2.70 mg/kg, i.p.

GSK334429

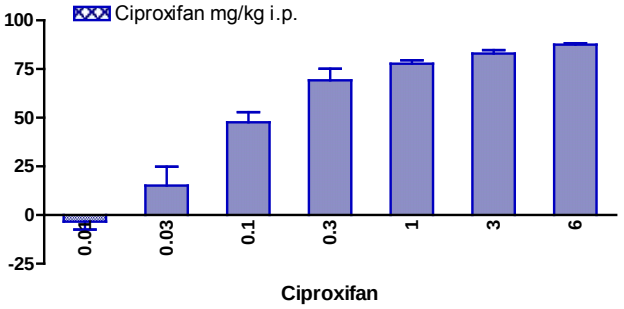
Ki = 5.9 nM



SUVEN ED_{50} = 0.14 mg/kg, p.o.
Reported ED_{50} = 0.35 mg/kg p.o.

Ciproxifan

Ki = 0.5 nM



SUVEN ED_{50} = 0.10 mg/kg, i.p.
Reported ED_{50} = 0.14 mg/kg, i.p.

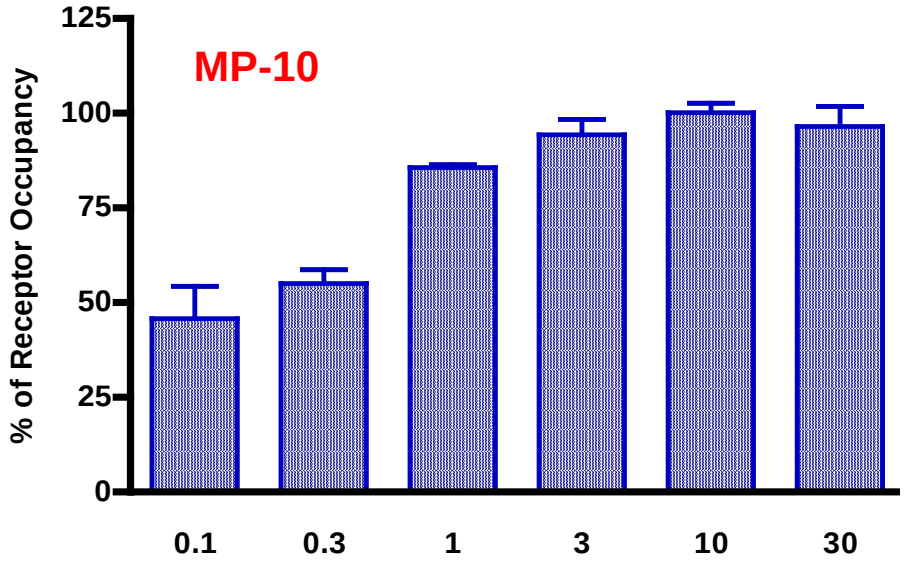
The calculated % receptor occupancy ED_{50} value from non-radiolabeled tracer is comparable to reported ED_{50} from study measured using radiolabeled tracer

JPET., 2008; Biochemical pharmacology., 2007

LC-MS/MS Based Receptor Occupancy

Phosphodiesterase 10 in Rats

Specific region: Striatum Non specific region: Thalamus Tracer: AMG 7980



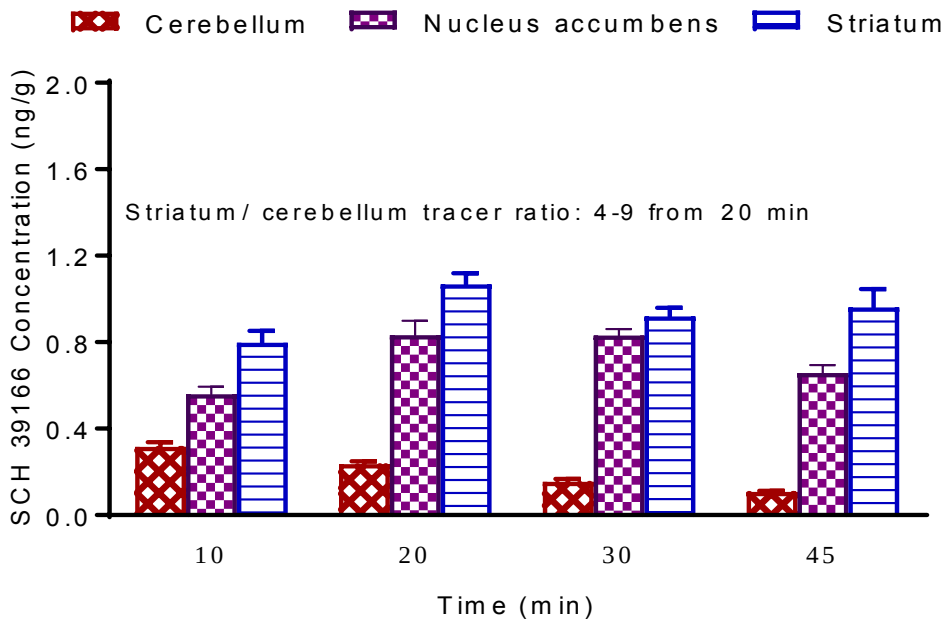
SUVEN ED_{50} = 0.57 mg/kg, p.o.
Reported ED_{50} = 2.3 mg/kg, p.o.

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.

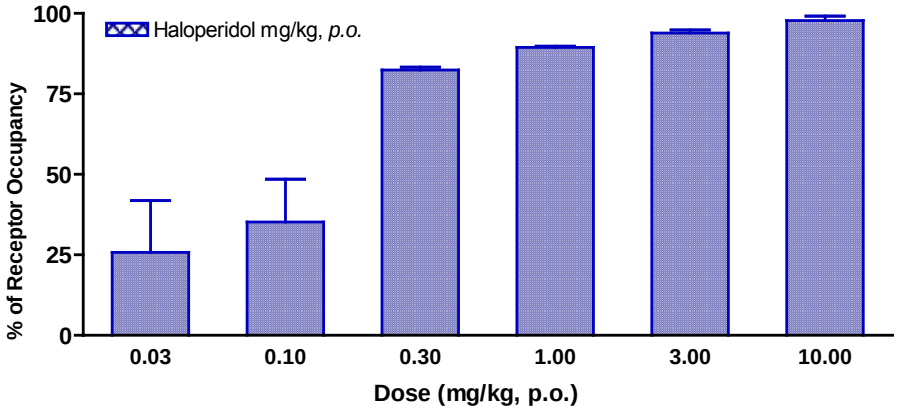
LC-MS/MS Based Receptor Occupancy

Dopamine D2 in Rats

Specific region: Striatum Non specific region: Cerebellum Tracer: Raclopride

Haloperidol

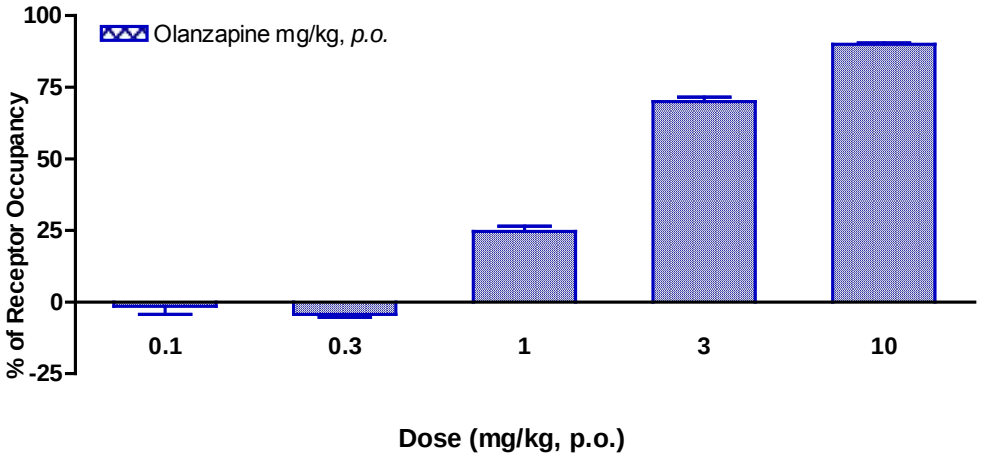
Ki = 1.4 nM



SUVEN ED_{50} = 0.12 mg/kg, p.o.
Reported ED_{50} = 0.20 mg/kg, p.o.

Olanzapine

Ki = 22.4 nM



SUVEN ED_{50} = 1.93 mg/kg, p.o.
Reported ED_{50} = 2.10 mg/kg, p.o.

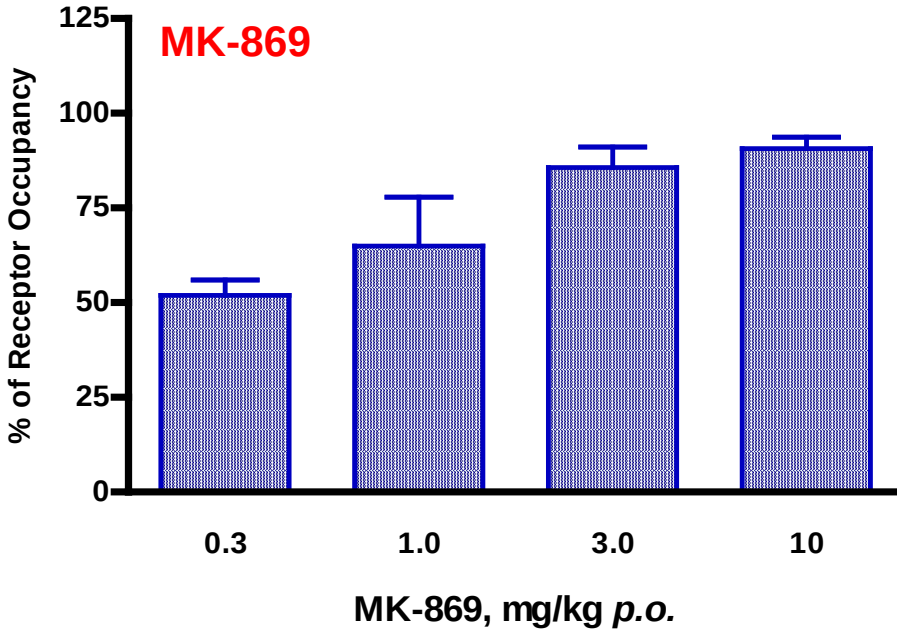
Life Sciences., 2005 and 2006.

The calculated % receptor occupancy ED_{50} value from non-radiolabeled tracer is comparable to reported ED_{50} 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



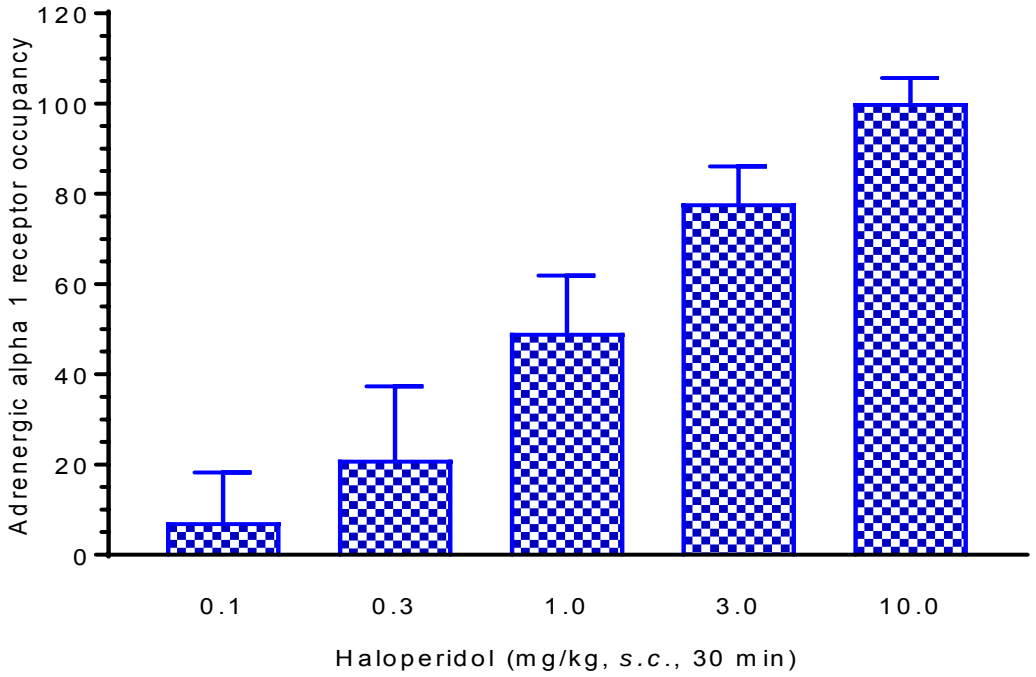
ED_{50} = 0.30 mg/kg, p.o.

LC-MS/MS Based Receptor Occupancy

Adrenergic alpha 1 in Rats

Specific region: Frontal cortex Non specific region: Cerebellum Tracer: Prazosin

Haloperidol



ED_{50} at Suven = 1.05 mg/kg, s.c.

LC-MS/MS Based Receptor Occupancy

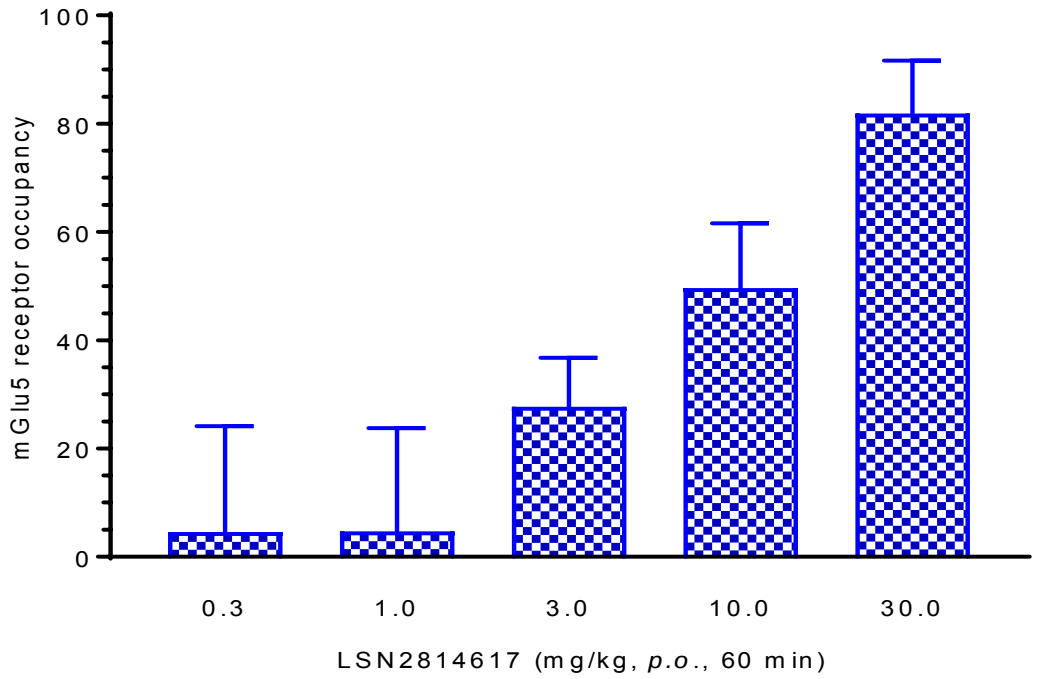
mGlu5 in Rats

Specific region: Striatum

Non specific region: Cerebellum

Tracer: mPEPy or ABP688

LSN2814617



ED_{50} at Suven = 8.81 mg/kg, *p.o.*

LC-MS/MS Based Receptor Occupancy

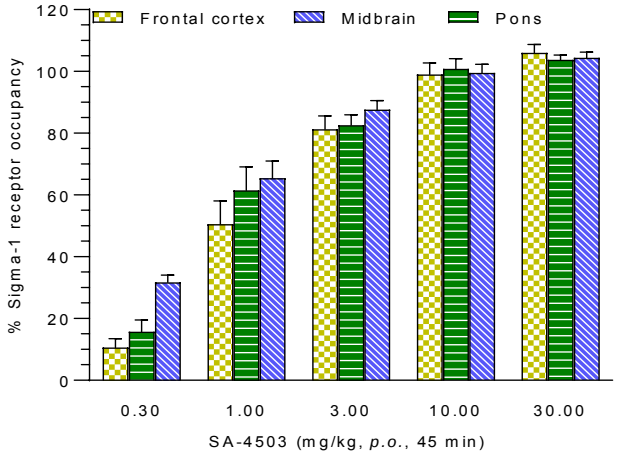
Sigma-1 in Rats

Specific region: Frontal cortex/ midbrain/ pons

Tracer: FTC-146

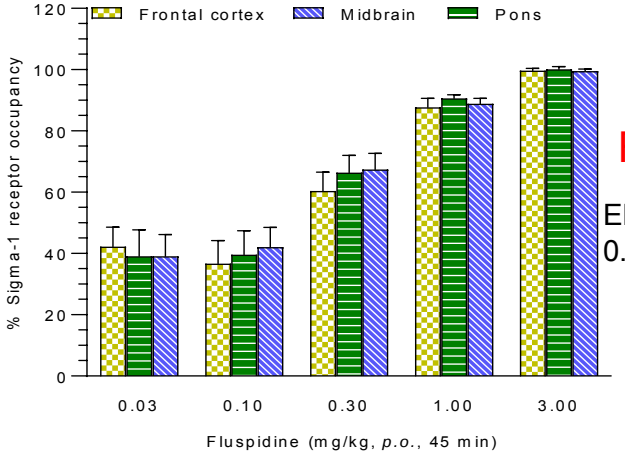
SA-4503

ED_{50} at Suven
0.58 - 1.04 mg/kg, *p.o*



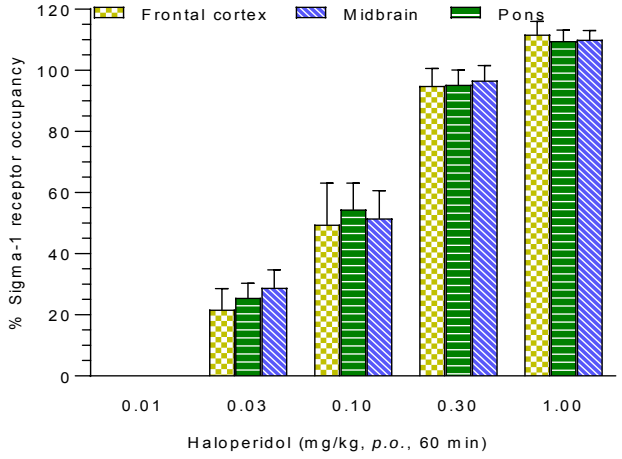
Fluspidine

ED_{50} at Suven
0.09 - 0.11 mg/kg, *p.o*



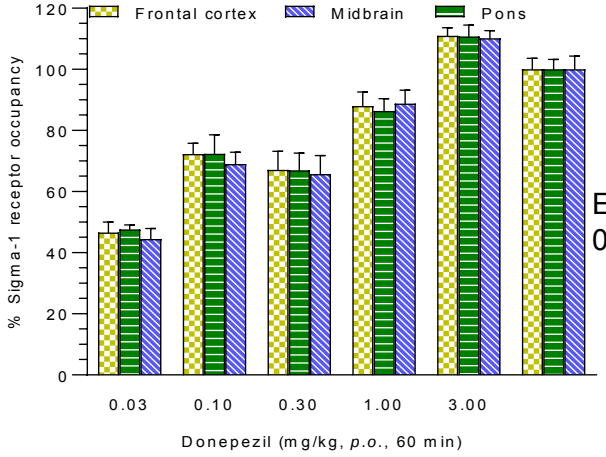
Haloperidol

ED_{50} at Suven
0.08 – 0.09 mg/kg, *p.o*



Donepezil

ED_{50} at Suven
0.035 – 0.043 mg/kg, *p.o*



LC-MS/MS Based Receptor Occupancy

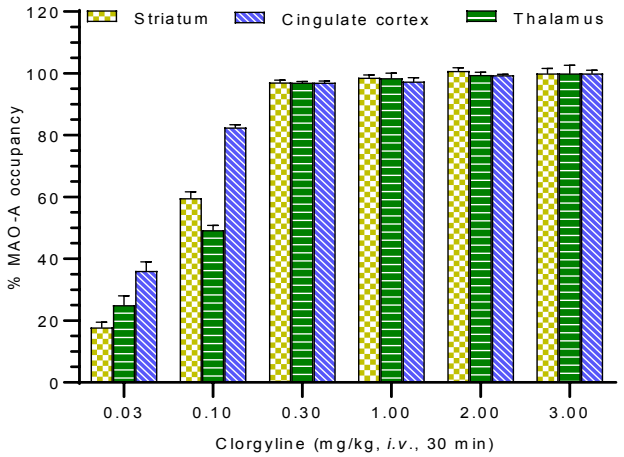
Monoamine oxidase (MAO)-A in Rats

Specific region: Striatum/ cingulate cortex/ thalamus

Tracer: Harmine

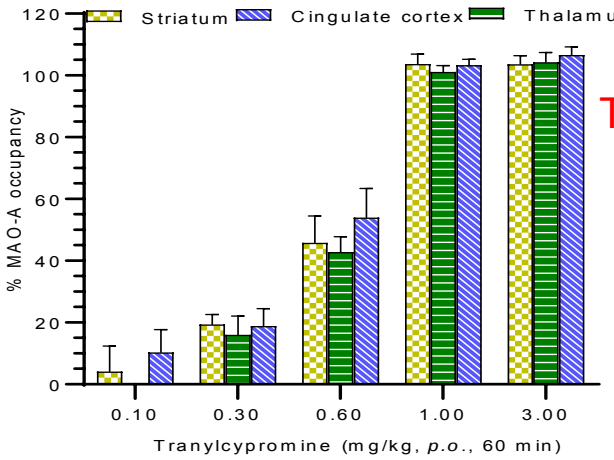
Clorgyline

ED_{50} at Suven
0.041 – 0.081 mg/kg, *i.v.*



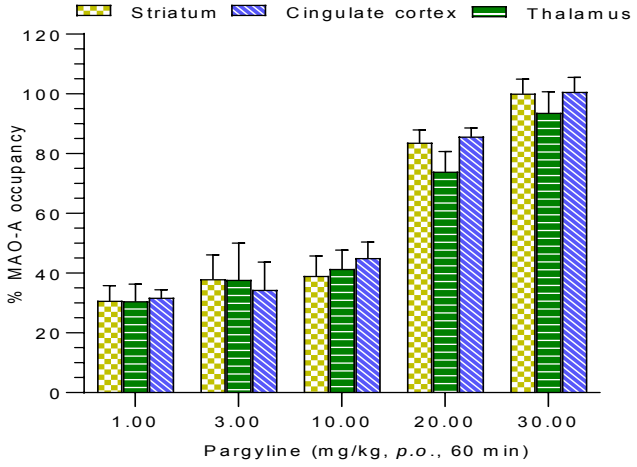
Tranylcypromine

ED_{50} at Suven
0.525 – 0.620 mg/kg, *p.o*



Pargyline

ED_{50} at Suven
5.0 – 5.6 mg/kg, *p.o*



LC-MS/MS Based Receptor Occupancy

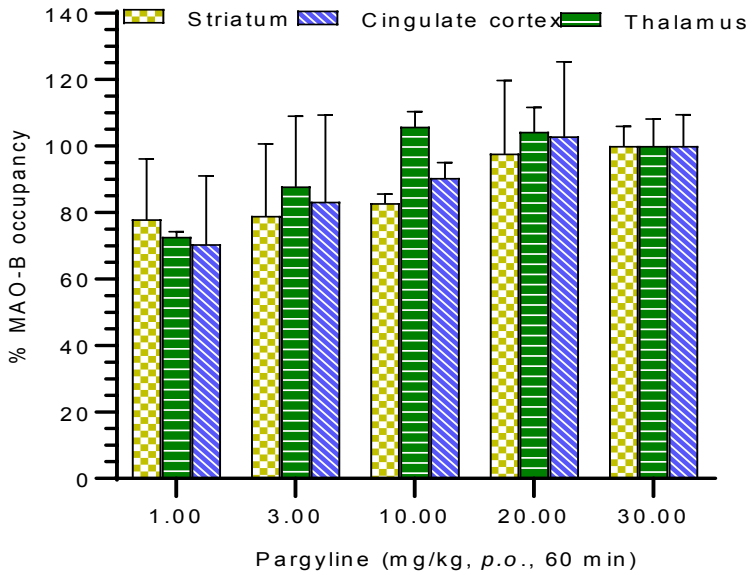
Monoamine oxidase (MAO)-B in Rats

Specific region: Striatum/ cingulate cortex/ thalamus

Tracer: R (-) Deprenyl

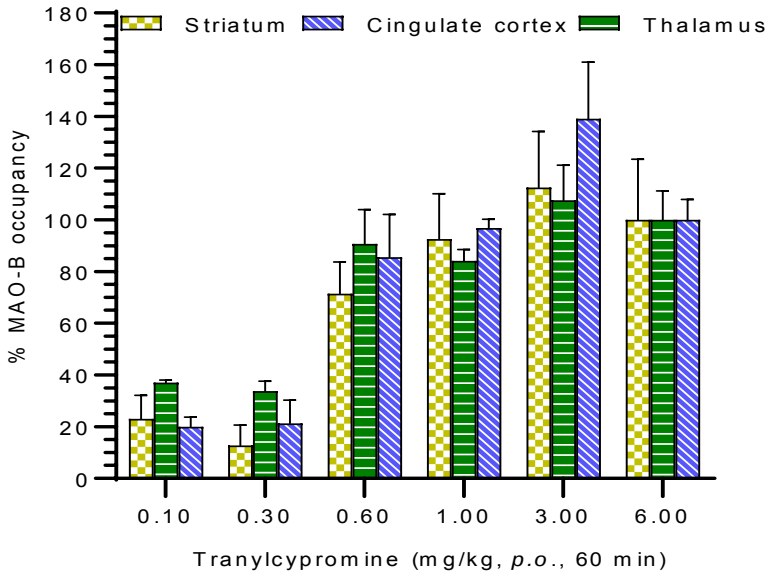
Pargyline

ED_{50} at Suven = 0.11 – 0.51 mg/kg, *p.o*



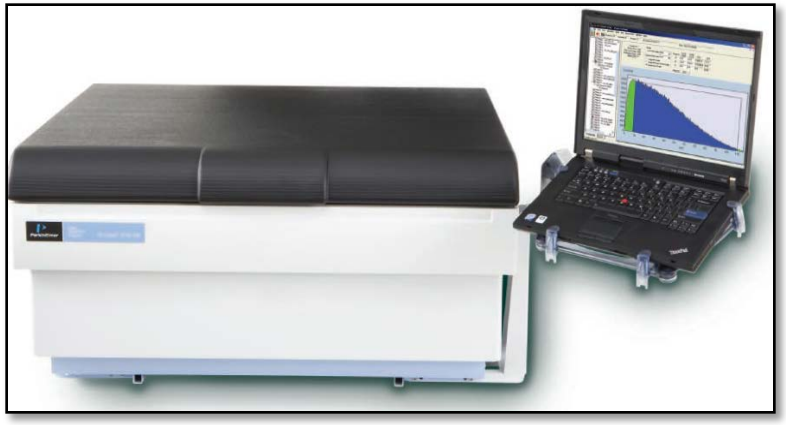
Tranylcypromine

ED_{50} at Suven = 0.25 – 0.48 mg/kg, *p.o*



Receptor occupancy using [3H] tracers

Overview



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

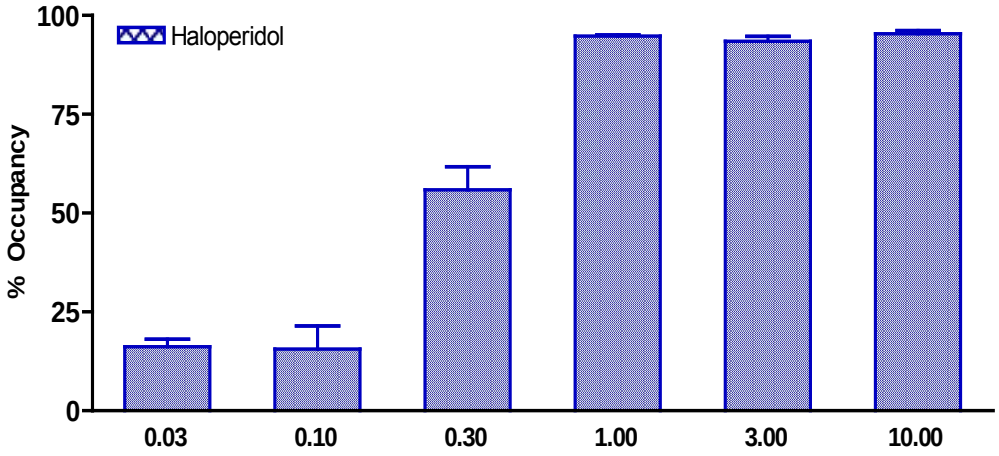
- **We have added Tricarb 3110TR* to our fleet of high-end instruments.**
- **This State- of- the- Art Technology Adds to Our Capability in Measurement of Pre-clinical Receptor Occupancy for Validated and Novel Targets**
- **Suven has Capability to Measure RO by Labeled and Non- Labeled Tracers**

*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: ^{3}H-Raclopride



RO ED_{50} = 0.25 mg/kg, p.o.

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

Contacts

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