The relationship between Psilocybin/psilocin plasma levels and receptor occupancy: PET imaging with 11C-Cimbi-36

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May 18th 2020

“It's tough to make predictions, especially about the future.”

-Yogi Berra

A Brief History of Psilocybin: From Isolation to Modern Clinical Trials

- In 1957 R. Gordon Wasson and Roger Heim collect *Psilocybe mexicana* fruiting bodies, mycelium and spores after being provided psilocybin-containing mushrooms. Shortly thereafter, Albert Hofmann isolates the active component from dried mushrooms provided by Roger Heim.

Psilocybe mexicana Heim (Phot. A. B.)

Fig. 1
Fruchtkörper
(auf künstlichem Nährboden, Abb. 1:1)

Fig. 2
Mycel mit Sklerotien
Kultur auf Nährlösung, Abb. 1:4,5)

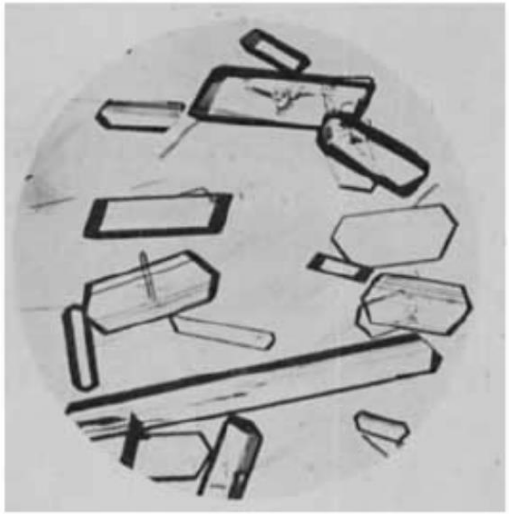


Fig. 3
Psilocybin
(aus Methanol)

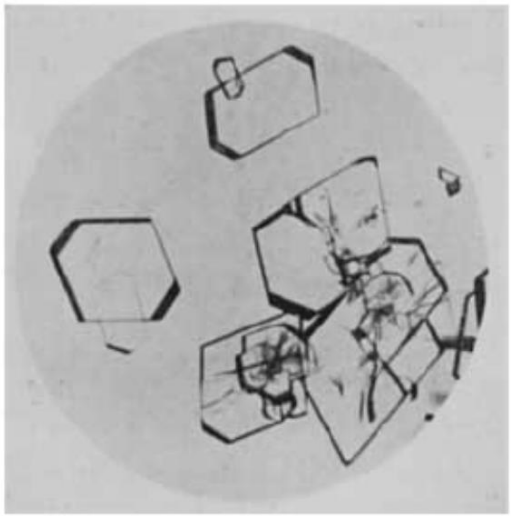
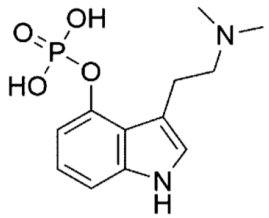
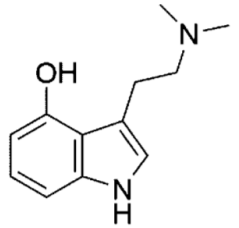


Fig. 4
Psilocin
(aus Methanol)



Psilocybin, **1**



Psilocin, **2**

Hofmann, A.; Heim, R.; Brack, A.; Kobel, H.; Frey, A.; Ott, H.; Petrzilka, T.; Troxler, F. Helv. Chim. Acta **1959**, 42, 1557– 1572

A Brief History of Psilocybin: From Isolation to Modern Clinical Trials

- In 1960 Sandoz introduces Indocybin (psilocybin) to psychiatry. Indocybin is discontinued by Sandoz (1966) and later designated as a controlled substance within the US (Controlled Substances Act, 1971) and the UN (Convention on Psychotropic Substances, 1971).

Commercial Sample of Psilocybin

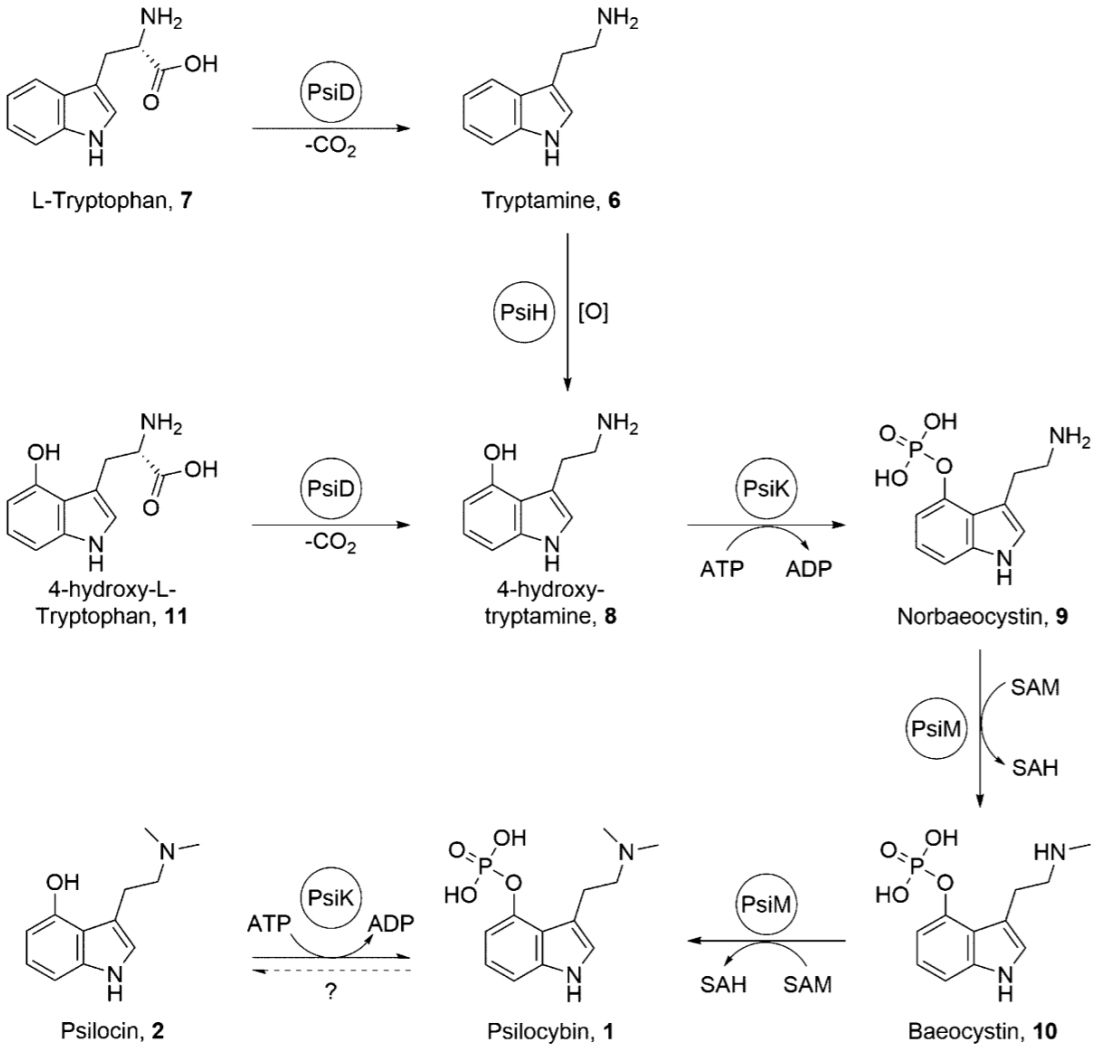
Spring Grove Hospital Center (Maryland)

- New government-funding (US) for psychedelic research ceases in 1970, with the last doses (dipropyltryptamine) administered as late as 1979 at Spring Grove.

Yensen R, Dryer D. **1992**. Thirty years of psychedelic research: the Spring Grove experiment and its sequels. Unpublished manuscript.

A Brief History of Psilocybin: From Isolation to Modern Clinical Trials

- Psilocybin is a secondary metabolite of tryptophan, produced naturally by a number of fungi (*Psilocybe c.).* Biosynthesis of Psilocybin:

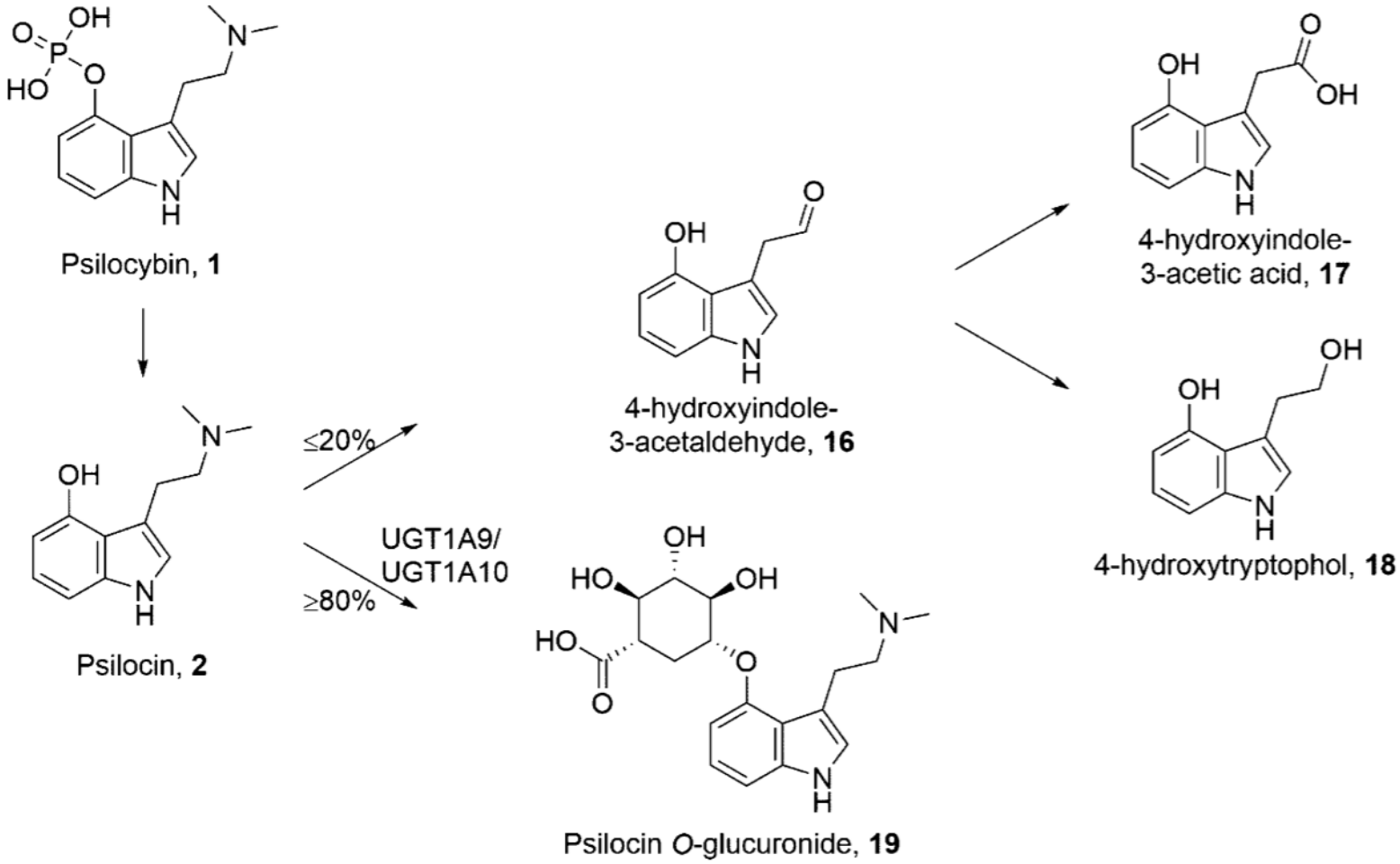


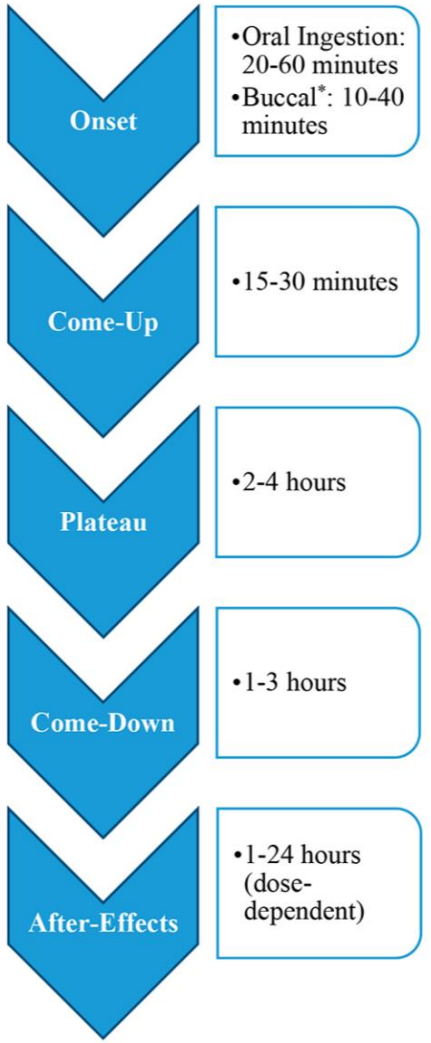
Psilocybe cubensis

Hofmann, A.; Heim, R.; Brack, A.; Kobel, H.; Frey, A.; Ott, H.; Petrzilka, T.; Troxler, F. Helv. Chim. Acta **1959**, 42, 1557– 1572

A Brief History of Psilocybin: From Isolation to Modern Clinical Trials

- Psilocybin (1) is not an active compound, but rather a prodrug. Metabolism (loss of phosphate group) leads to the *in vivo* production of Psilocin (2, active). The subjective effects of Psilocin generally last from 3-7h post ingestion.

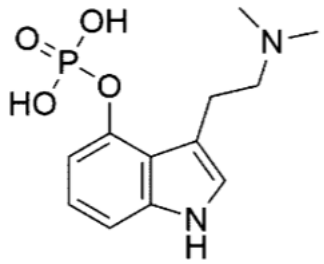




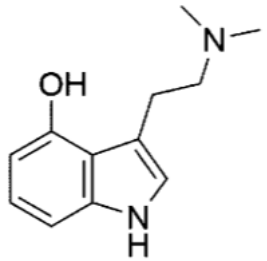
Hofmann, A.; Heim, R.; Brack, A.; Kobel, H.; Frey, A.; Ott, H.; Petrzilka, T.; Troxler, F. Helv. Chim. Acta **1959**, 42, 1557– 1572

A Brief History of Psilocybin: From Isolation to Modern Clinical Trials

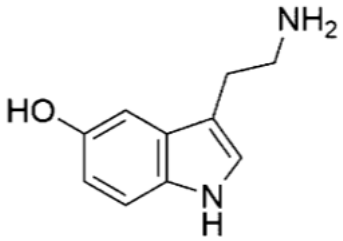
- Psilocin displays "polypharmacology," complicating mechanism-of-action studies.



Psilocybin, **1**



Psilocin, **2**



Serotonin, **3**

K_i of Psilocin at Major Receptors

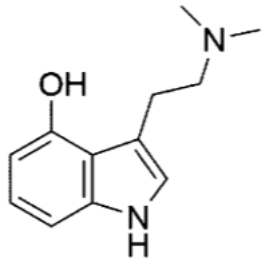
binding site	K_i (nM)	binding site	K_i (nM)
SERT	3801	α_{1A}	>10 000
5-HT_{1A}	567.4	α_{1B}	>10 000
5-HT_{1B}	219.6	α_{2A}	1379
5-HT_{1D}	36.4	α_{2B}	1894
5-HT_{2A}	107.2,[23] 25	α_{2C}	>10 000
5-HT_{2B}	4.6	β_1	>10 000
5-HT_{2C}	97.3	D_1	>10 000
5-HT_3	>10 000	D_2	>10 000
5-HT_5	83.7	D_3	2645
5-HT_6	57.0	D_4	>10 000
5-HT_7	3.5	D_5	>10 000
H_1	304.6		

- Ki values for Psilocin at 5HT2a receptor determined with agonist- or antagonist-radioligands, respectively.

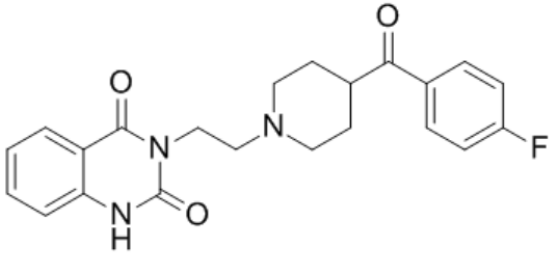
Hofmann, A.; Heim, R.; Brack, A.; Kobel, H.; Frey, A.; Ott, H.; Petrzilka, T.; Troxler, F. Helv. Chim. Acta **1959**, 42, 1557– 1572

A Brief History of Psilocybin: From Isolation to Modern Clinical Trials

- The psychedelic effects of Psilocin are thought to be derived primarily from agonist activity at the 5HT2a receptor. This is supported by several human trials demonstrating that the subjective effects of Psilocybin are blocked following pre-administration of ketanserin.



Psilocin, **2**



Ketanserin
(selective 5HT2a antagonist)
5HT2a Ki = 2 nM
5HT2c Ki = 130 nM

commonly reported effects of psilocybin ingestion	
physiologic effects	mild sedation with compulsive yawning; stimulation; physical euphoria; feelings of weightlessness; tactile enhancement; rhinorrhea; mydriasis; hypersalivation; increased systolic pressure; slight elevation in body temperature
visual effects	enhancement: color saturation; pattern recognition; visual acuity (at lower doses)
	distortions: flowing/breathing/melting of objects and colors; tracers; perspective distortion
	hallucinations: bright and colorful shapes and figures seen with eyes closed and with eyes open at higher doses
cognitive effects	increased empathy; simultaneous emotions; enhanced objective and situational analysis; music appreciation; ego loss; catharsis; rejuvenation; addiction suppression; time distortion
auditory effects	sound enhancement and distortion
multisensory effects	synesthesia
transpersonal effects	increased spirituality and a sense of interconnection between humanity and a higher power

ACS Chem. Neurosci. **2018**, 9, 2438−2447

For a comprehensive Review See: Nichols DE, *Pharmacological Reviews* **2016**, 68, 264-355

A Brief History of Psilocybin: From Isolation to Modern Clinical Trials

1994

DMT

Dose Respose

(60 patients)

2006

Psilocybin

0.1, 0.2, 0.3 mpk PO

OCD

(9 patients)

THE UNIVERSITY OF ARIZONA

2011

Psilocybin

0.2 mpk PO

Existential Distress

(12 patients)

2014

Psilocybin

0.28, 0.42 mpk PO

Smoking Cessation

(15 patients)

JOHNS HOPKINS UNIVERSITY

2015

Psilocybin

0.3, 0.4 mpk PO

Alcohol Use Disorder

(10 patients)

2016

Psilocybin

10, 20 mg PO

Treatment Resistance Depression

Imperial College London

2016

Psilocybin

0.3 mpk PO

Existential Distress

(29 patients)

NYU

2016

Psilocybin

0.28, 0.42 mpk PO

Existential Distress

(51 patients)

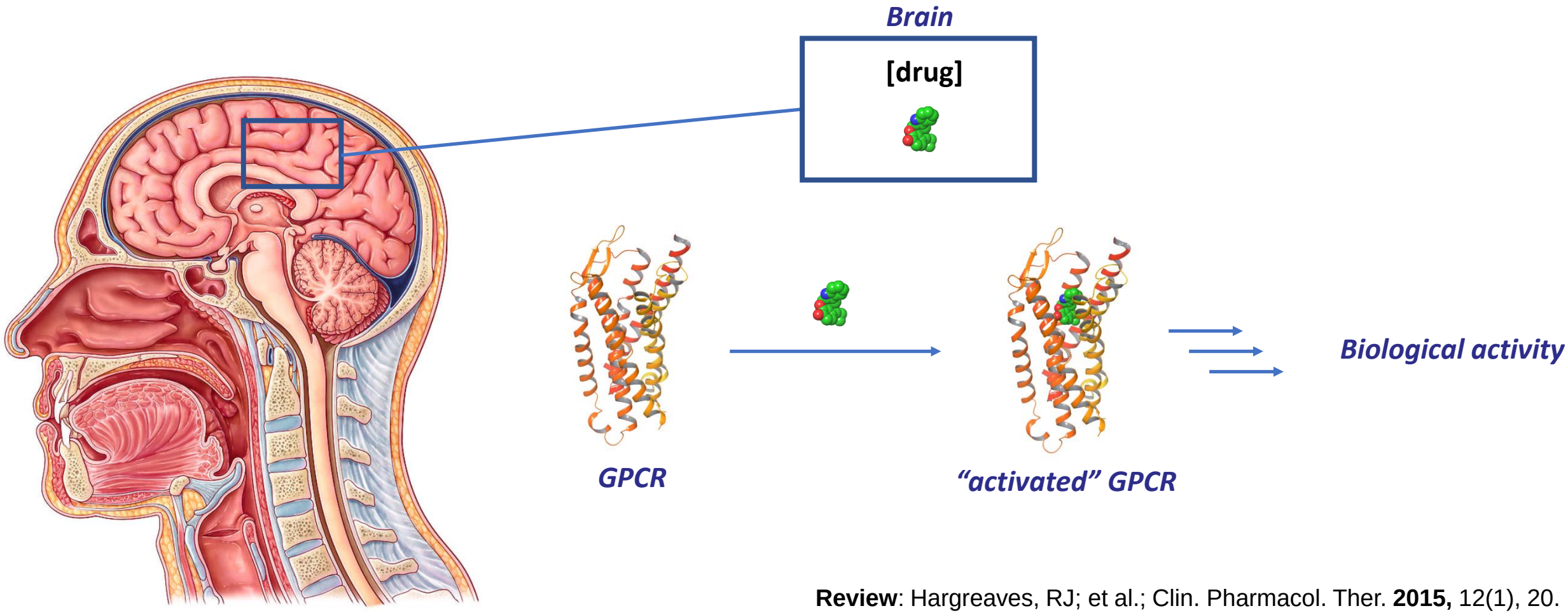
JOHNS HOPKINS UNIVERSITY

= double-blind, randomized, placebo-controlled

= open-label with or w/out placebo

PET Receptor Occupancy Studies in CNS Drug Discovery: Basic Concepts

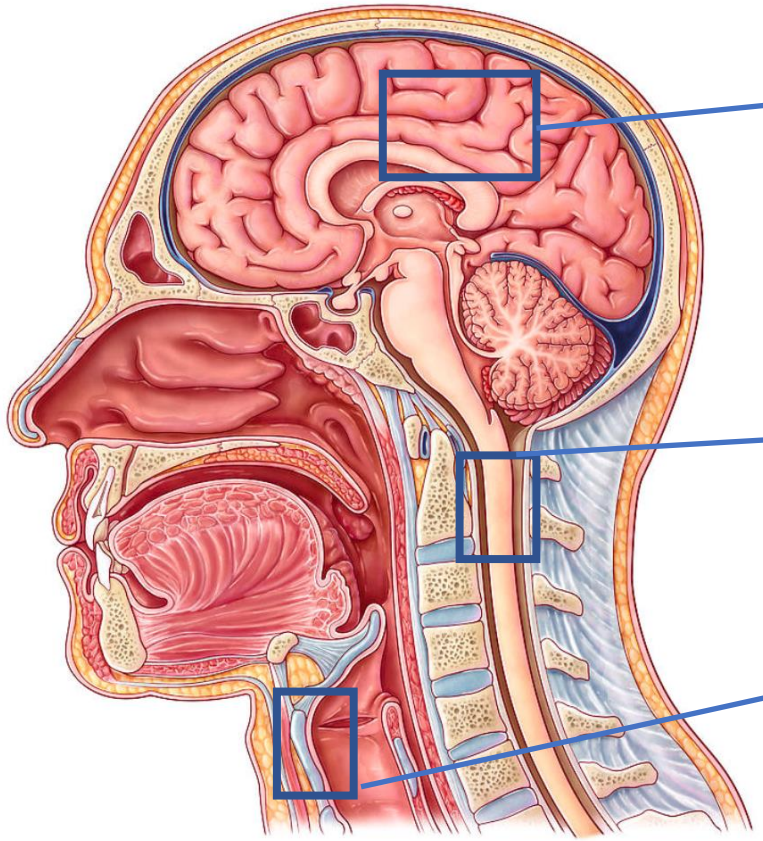
- The most important pharmacokinetic parameter for all centrally-acting therapeutics is unbound-brain concentration of drug.
  - This feature governs target engagement and occupancy and is related to pharmacodynamic effect.

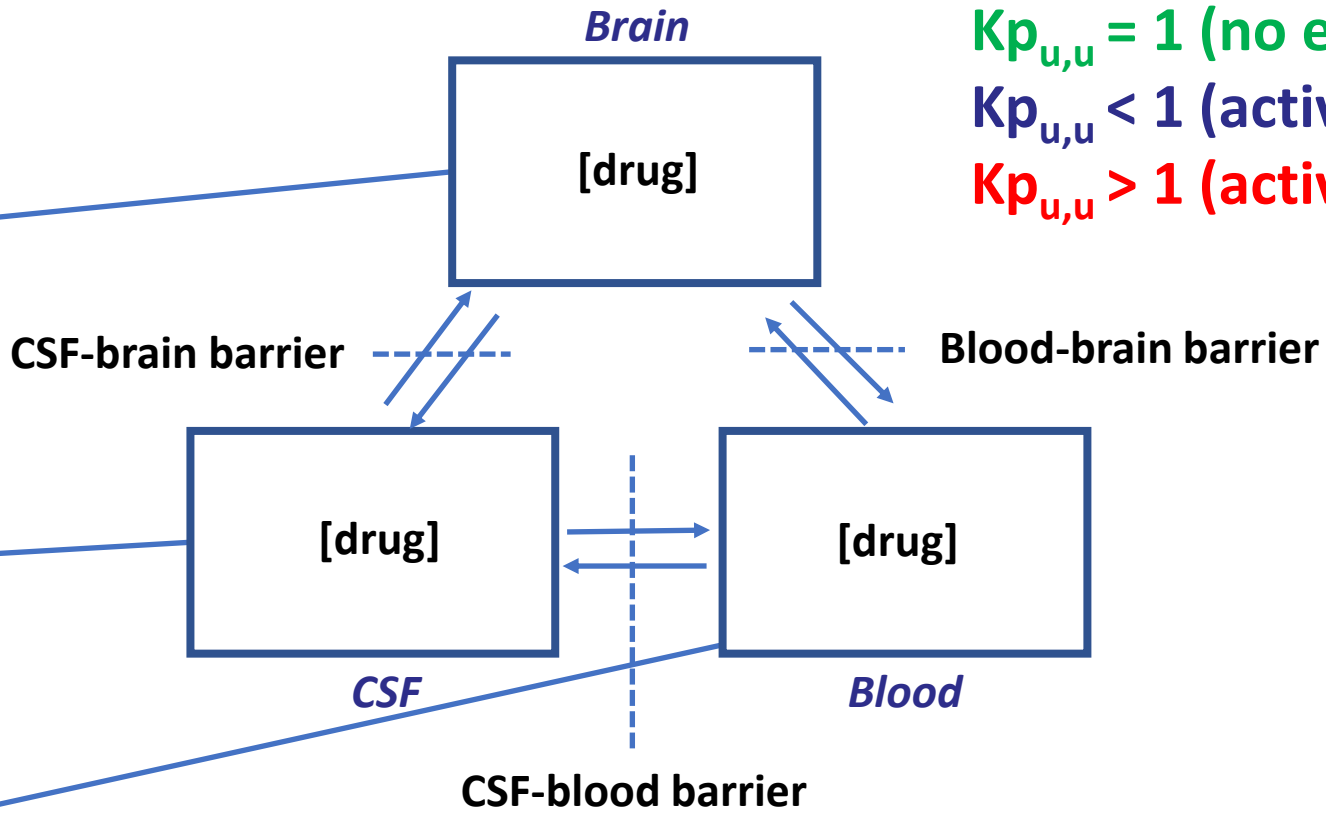


Review: Hargreaves, RJ; et al.; Clin. Pharmacol. Ther. **2015,** 12(1), 20.

PET Receptor Occupancy Studies in CNS Drug Discovery: Basic Concepts

- Following peripheral administration (PO, IV, etc), a small molecule can distribute throughout the body. The unbound brain:plasma ($Kp_{u,u}$) ratio will approach unity with adjacent compartments (Blood, CSF), assuming it **1) lacks active transport via efflux pumps (PGP, BCRP) or influx (rare) 2) is freely membrane permeable 3) penetration has reached steady-state.**





$Kp_{u,u}$ = 1 (no efflux)
$Kp_{u,u}$ < 1 (active efflux)
$Kp_{u,u}$ > 1 (active transport)

Detailed Review: *Pharmaceutics* **2020,** 12(1), 20.

PET Receptor Occupancy Studies in CNS Drug Discovery: Basic Concepts

- Following peripheral administration (PO, IV, etc), a small molecule can distribute throughout the body. The unbound brain:plasma ($Kp_{u,u}$) ratio will approach unity with adjacent compartments (Blood, CSF), assuming it **1) lacks active transport via efflux pumps (PGP, BCRP) or influx (rare) 2) is freely membrane permeable 3) penetration has reached steady-state**

Example Drug # 1

No pgp/BCRP liability

No active influx

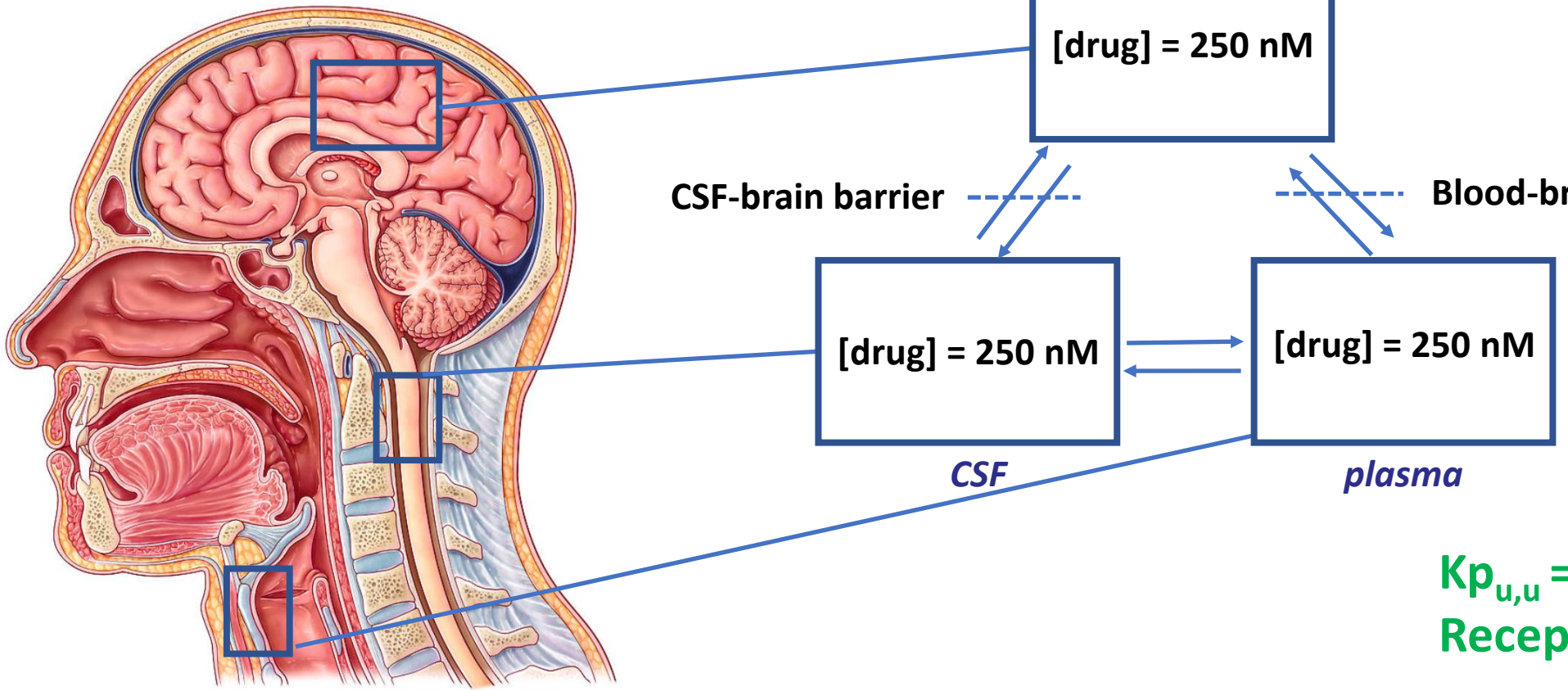
C_{max} = 500 nM (plasma)

T_{max} = 1 hr

f_{plasma} = 0.5

f_{brain} = 0.25

K_D = 250 nM



$Kp_{u,u}$ = 1

Receptor occupancy = 50%

PET Receptor Occupancy Studies in CNS Drug Discovery: Basic Concepts

- Following peripheral administration (PO, IV, etc), a small molecule can distribute throughout the body. The unbound brain:plasma ($Kp_{u,u}$) ratio will approach unity with adjacent compartments (Blood, CSF), assuming it **1) lacks active transport via efflux pumps (PGP, BCRP) or influx (rare) 2) is freely membrane permeable 3) penetration has reached steady-state**

Example Drug # 2

PGP ER > 30

No active influx

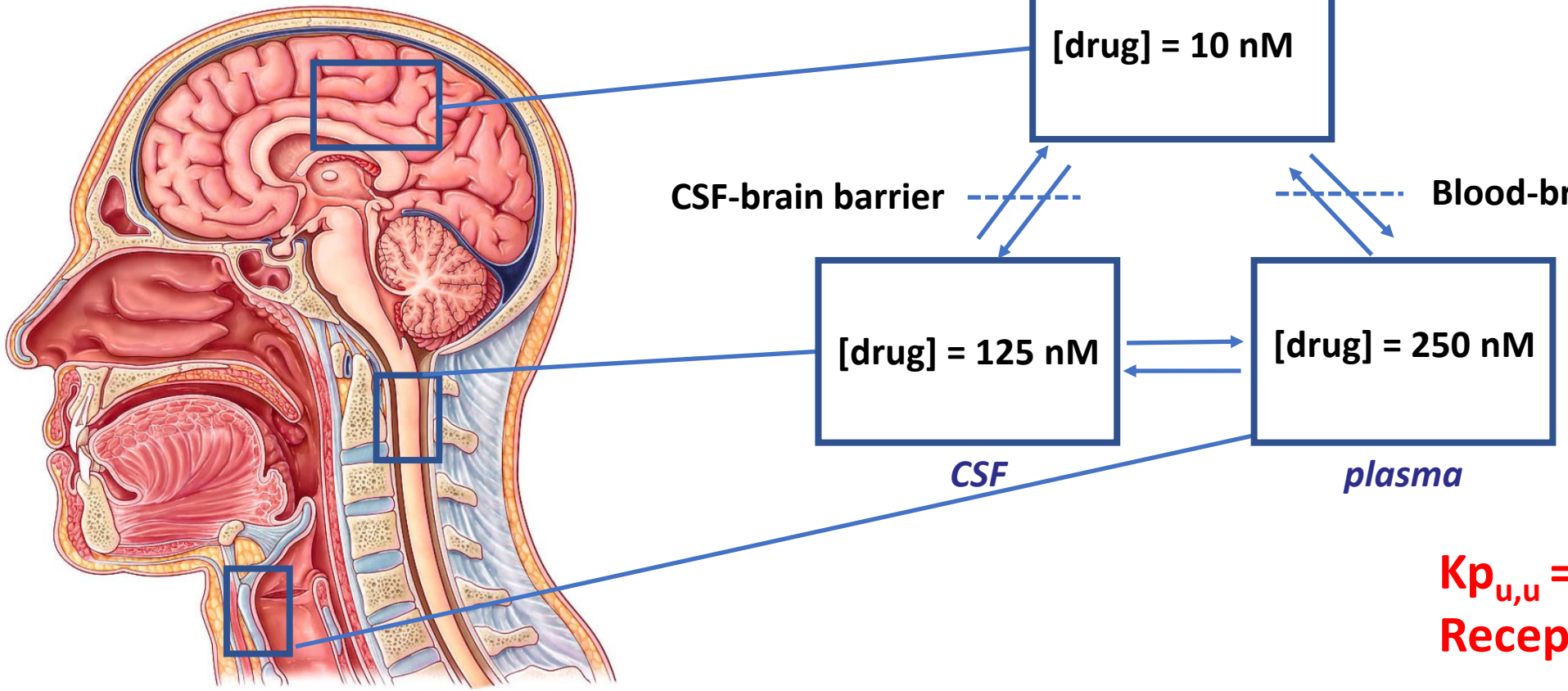
C_{max} = 500 nM (plasma)

T_{max} = 1 hr

f_{plasma} = 0.5

f_{brain} = 0.25

K_D = 250 nM



$Kp_{u,u}$ = < 0.1

Receptor occupancy = <5%

PET Receptor Occupancy Studies in CNS Drug Discovery: Basic Concepts

- **Unbound concentration of drug within the brain can not be determined directly in humans!**
  - **All pre-clinical methods require terminal studies (this is costly and impractical with NHPs).**

- **Species differences in efflux transporter concentration can make translation of $Kp_{u,u}$ afrom pre-clinical species a risky proposition.**

TABLE 1

Protein expression of MDR1 (P-glycoprotein) and BCRP across species

	Mouse[a]	Rat[b,c]	NHP[d]	Human[a]
MDR1 (fmol/μg)	14.1	19.1	4.71	6.06
BCRP (fmol/μg)	4.41	4.95	14.2	8.14

BCRP, breast cancer resistance protein; MDR1, multidrug resistance protein 1; NHP, nonhuman primate.

TABLE 3

Percentage of predictions within 2-fold of observed for each of the scaling factor (proteomic REF and parameter estimate RAF) sets, with and without $f_{u,b}$ correction

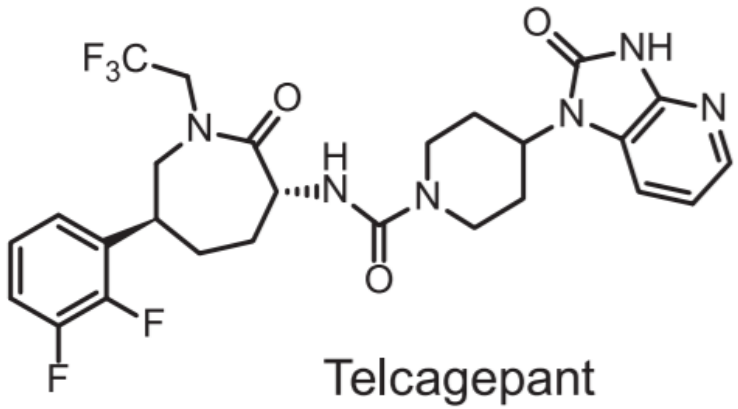
Animal	Proteomics		Parameter Estimate	
	$f_{u,b}$	$f_{u,b\ cor}$	$f_{u,b}$	$f_{u,b\ cor}$
Mouse	72%	77%	66%	67%
Rat	65%	72%	76%	71%
NHP	93%	73%	87%	60%

$f_{u,b}$, fraction unbound in brain; $f_{u,b,cor}$, corrected fraction unbound in brain; NHP, nonhuman primate; RAF, relative activity factor; REF, relative expression factor.

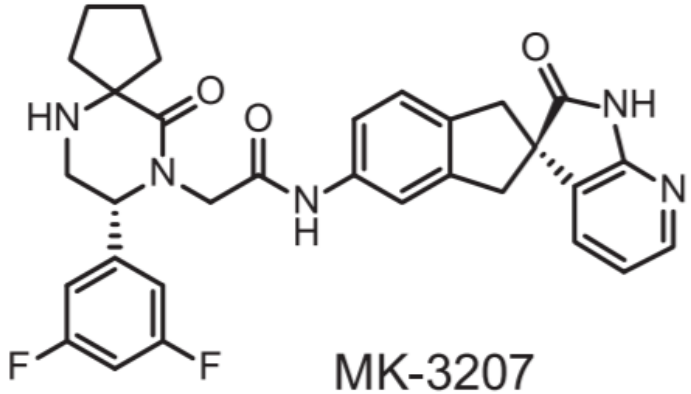
Trapa, P.E.; et al. *Drug Metab. Dispos.* **2019,** 47, 405–411

PET Receptor Occupancy Studies in CNS Drug Discovery: Basic Concepts

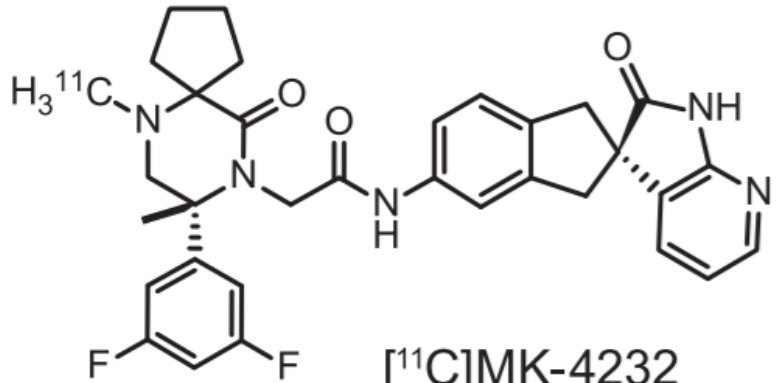
- PET imaging enables the direct assessment of receptor occupancy.
  - Use of the CGRP PET imaging agent [^{11}C]MK-4232 enabled the direct assessment of CGRP-R occupancy, confirming that central target engagement was not required for anti-migraine effects.



Telcagepant
K_i = 0.77 nM
P-gp = 24



MK-3207
K_i = 0.021 nM
P-gp = 25



[^{11}C]MK-4232
K_i = 0.039 nM
P-gp = 1.7

Hostetler, ED; et al.; J. Pharmacol Exp. Ther. **2013,** 347, 478.

PET Receptor Occupancy Studies in CNS Drug Discovery: Basic Concepts

- Autoradiography is utilized to validate and confirm the origin of the *in vivo* PET signal.

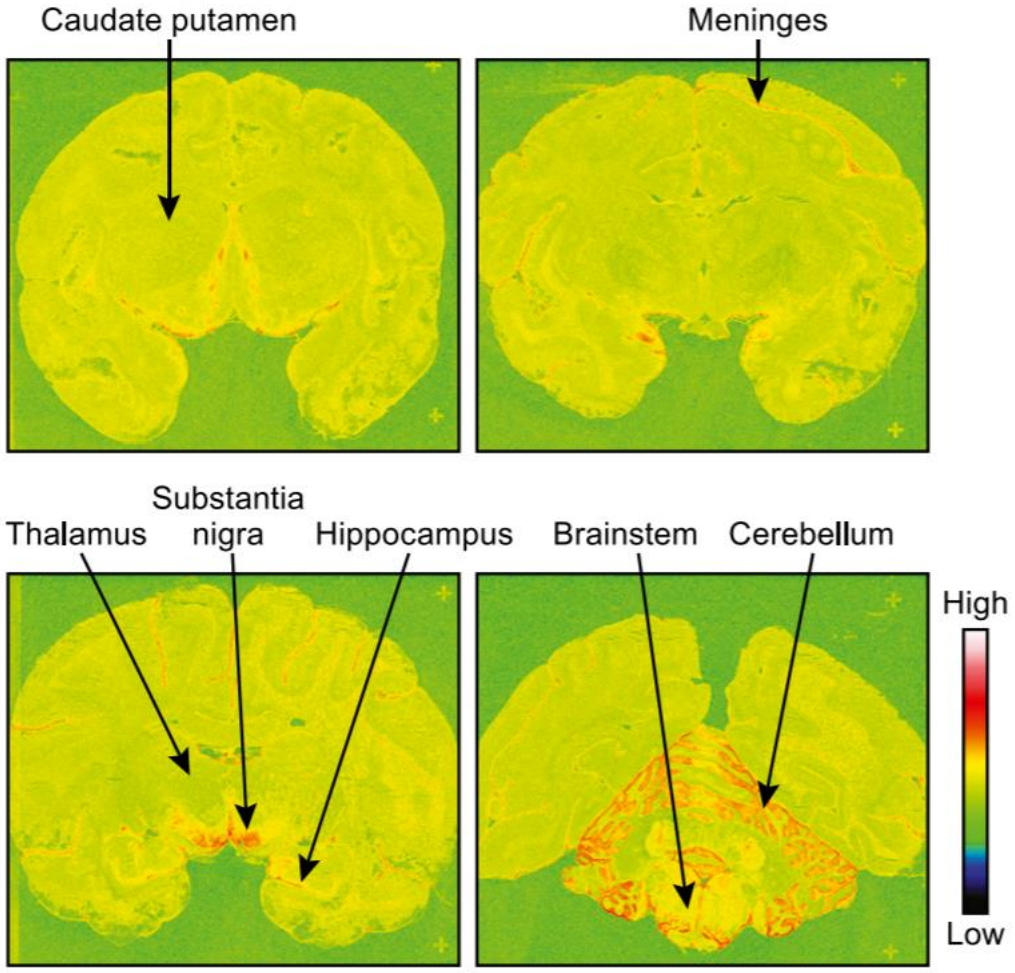


Fig. 2. [^{3}H]MK-4232 in vitro autoradiography of rhesus monkey brain slices.

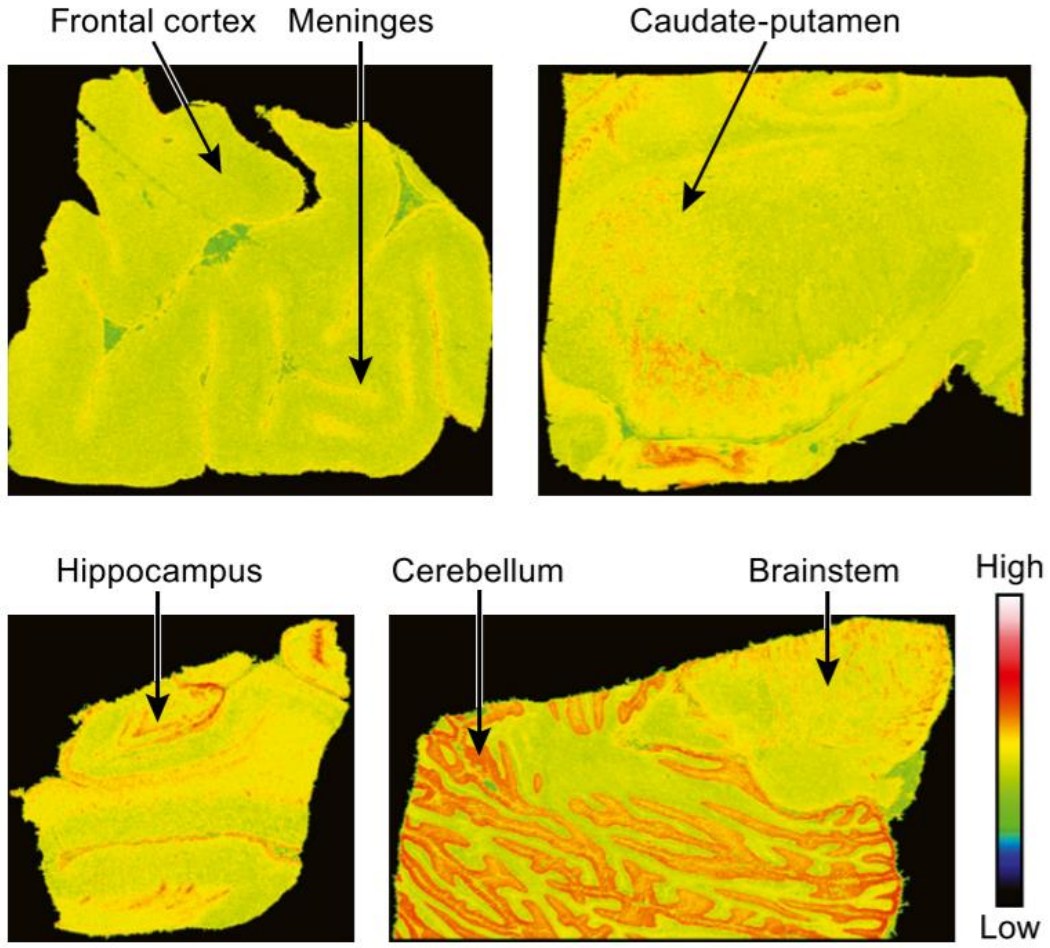
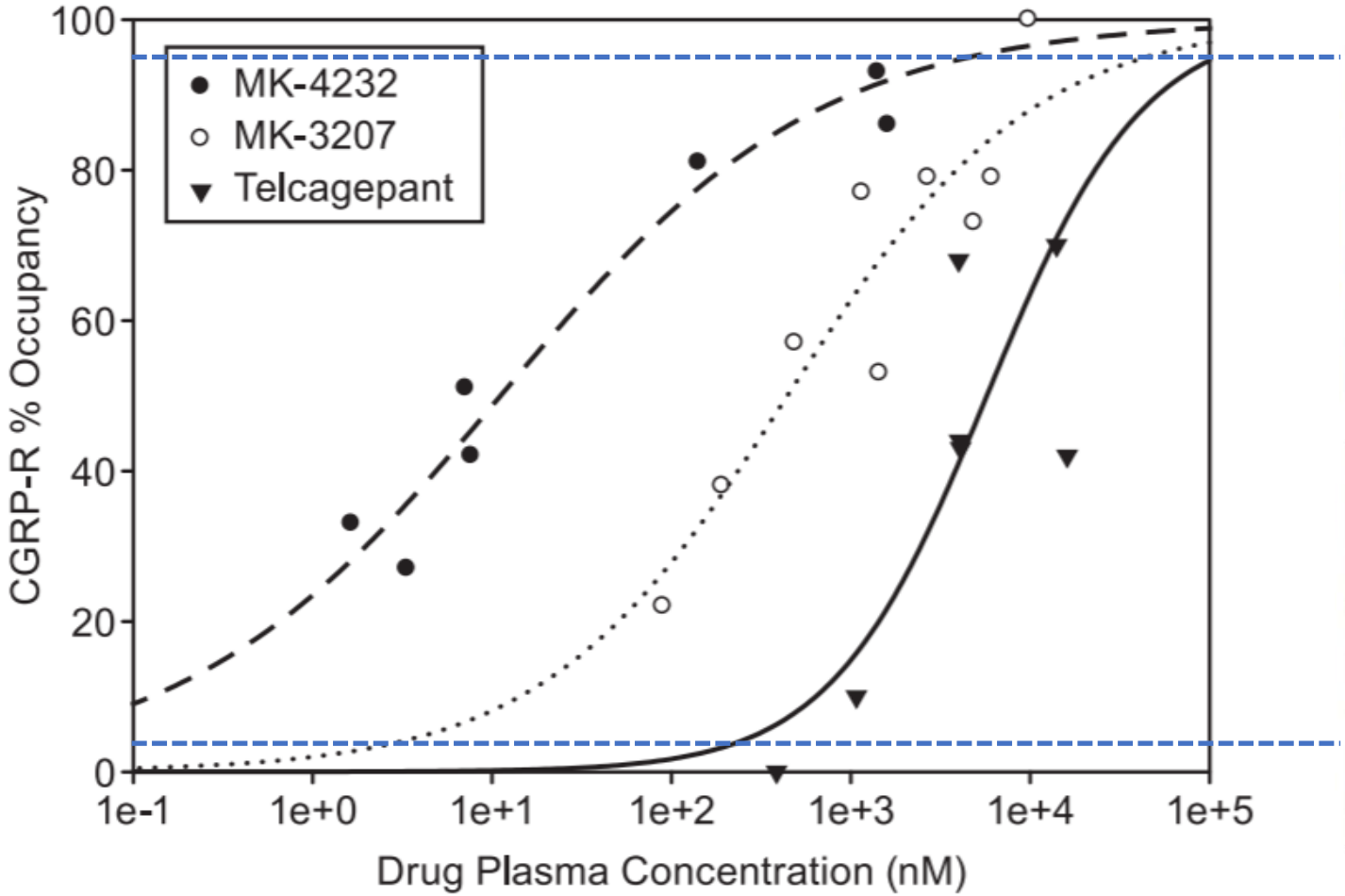


Fig. 3. [^{3}H]MK-4232 in vitro autoradiography of human brain slices.

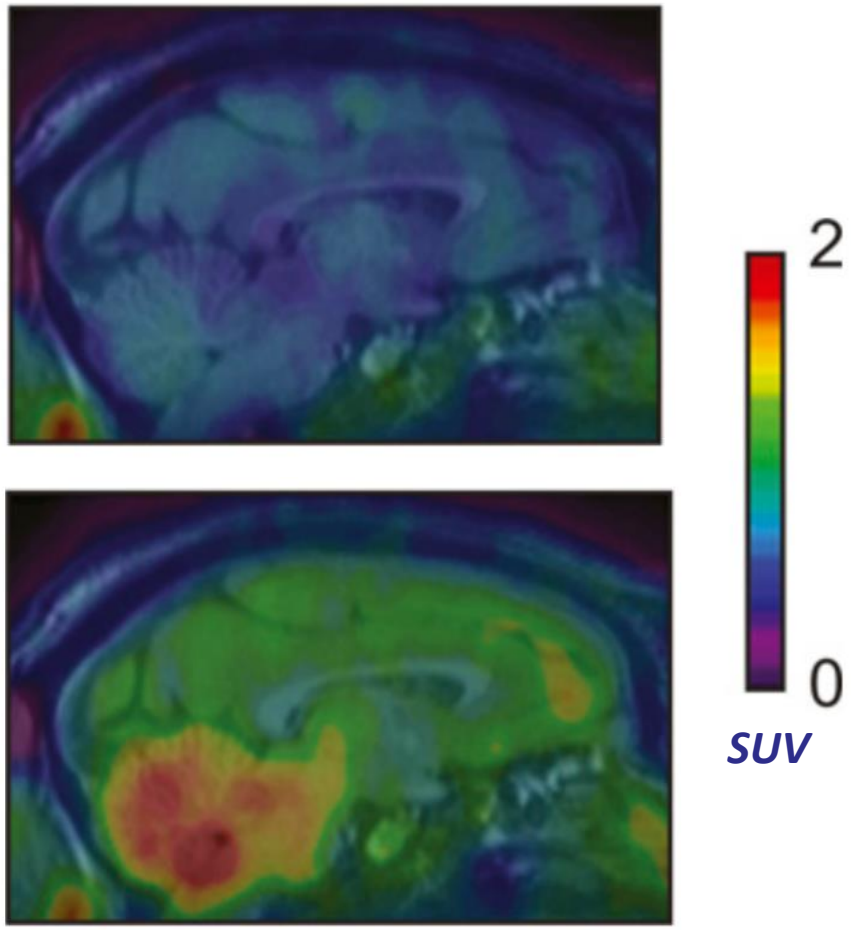
Hostetler, ED; et al.; J. Pharmacol Exp. Ther. **2013,** 347, 478.

PET Receptor Occupancy Studies in CNS Drug Discovery: Basic Concepts

- Interpretation of RO curves (Rhesus monkey RO curve and images):



High Occupancy >90%



Baseline (low occupancy <5%)

MK-4232 Ki = 0.039 nM (non-pgp substrate)

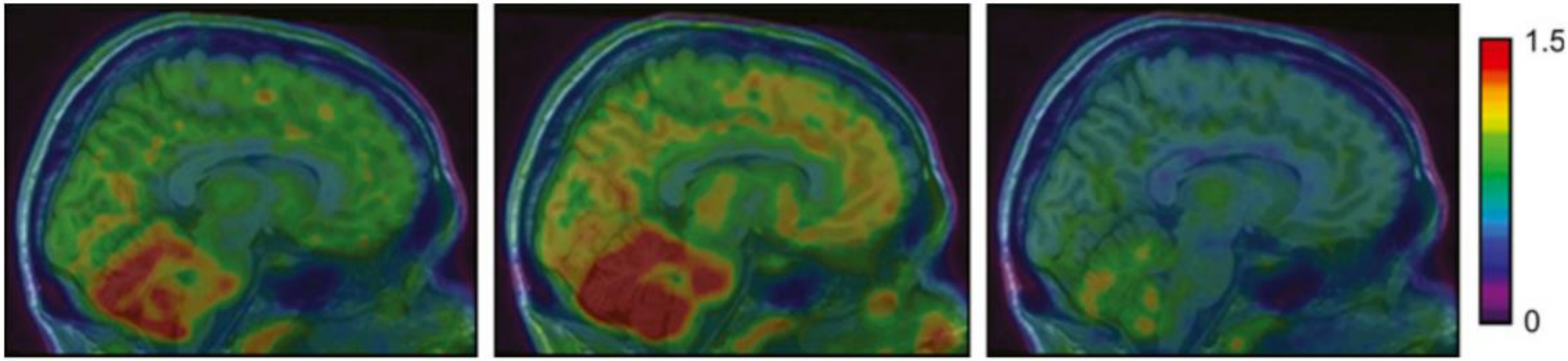
MK-3207 Ki = 0.021 Telc. Ki = 0.77 nM (pgp substrates)

Hostetler, ED; et al.; J. Pharmacol Exp. Ther. **2013,** 347, 478.

PET Receptor Occupancy Studies in CNS Drug Discovery: Basic Concepts

- Interpretation of RO curves (Human images):
  - Central engagement of CGRP-R is not needed for efficacy!

Baseline ***140 mg*** ***1120 mg***

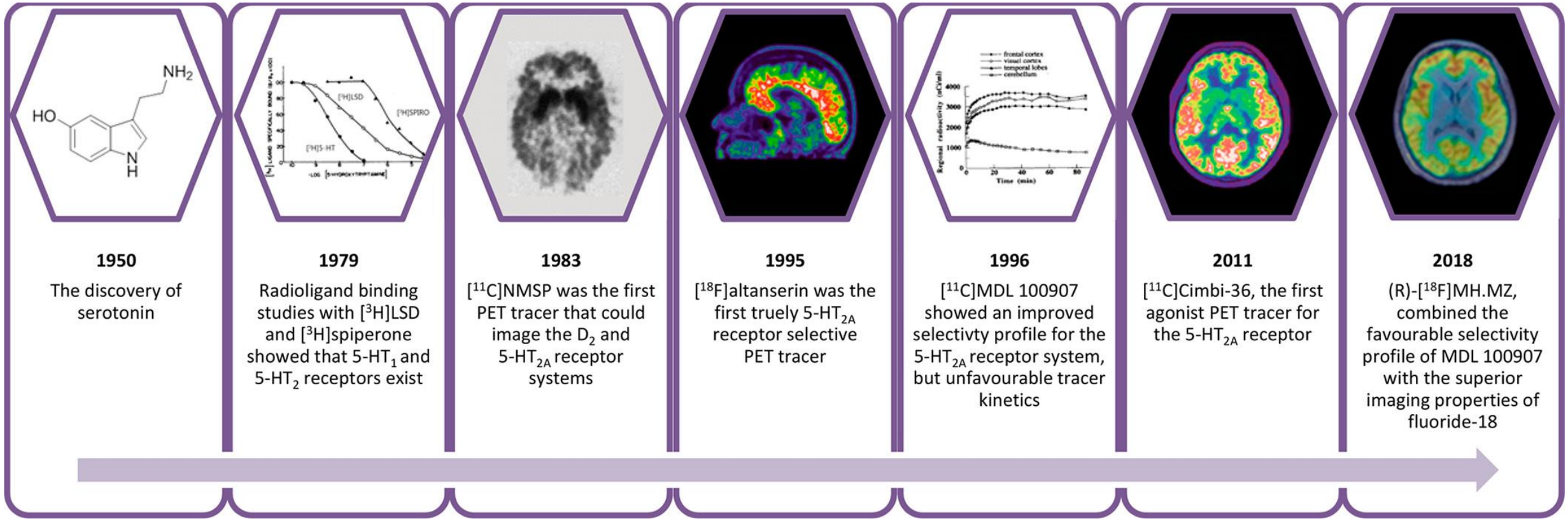


Subject	Telcagepant Dose	Telcagepant Plasma Concentration	CGRP-R Occupancy
	mg	*μM*	*%*
1	1120	16.3 ± 1.84	43
7	1120	20.2 ± 3.93	48
8	1120	22.2 ± 4.43	58
9	140	0.254 ± 0.140	5
1	140	0.859 ± 0.305	10
10	140	0.424 ± 0.187	4

Hostetler, ED; et al.; J. Pharmacol Exp. Ther. **2013,** 347, 478.

A Brief History of Molecular Imaging at the 5HT2a receptor

- Molecular Imaging at the 5HT2a receptor:

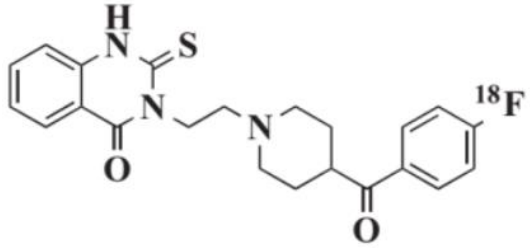


- Significant work has been accomplished with fMRI within this area; see: Carhart-Harris RL, et al. **2012** *Proc Natl Acad Sci* 109, 2138–2143.

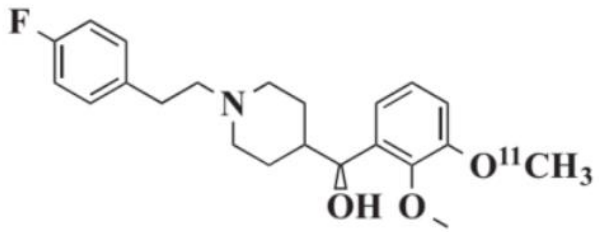
ACS Chem. Neurosci. **2018**, 9, 6, 1226-1229.

PET imaging at the 5HT2a receptor

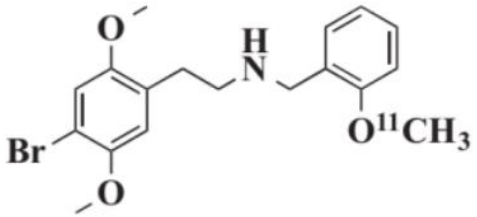
- *In vitro* comparison of select 5HT2a probes:



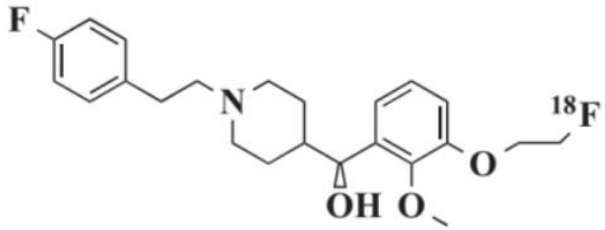
[^{18}F]altanserin



[^{11}C]MDL 100907



[^{11}C]Cimbi-36



(R)-[^{18}F]MH.MZ

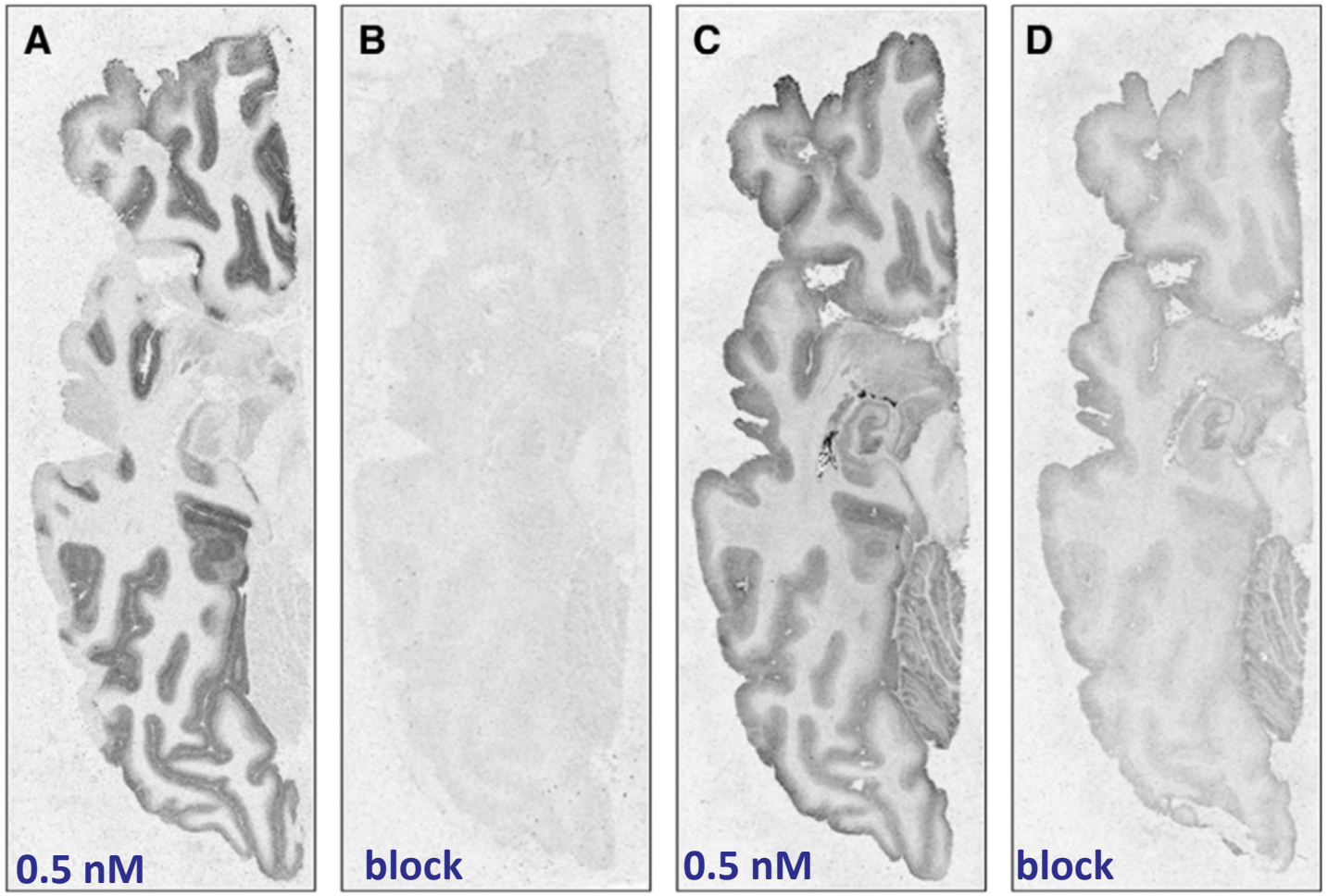
	5-HT$_{2A}$	5-HT$_{2C}$	5-HT$_{1A}$	D$_2$	α_1
MDL 100907	0.36 nM	107 nM	> 10.000 nM	2250 nM	128 nM
(R)-MH.MZ	0.72 nM	53 nM	> 10.000 nM	2686 nM	335 nM
altanserin	0.13 nM	6 nM	1570 nM	62 nM	4.55 nM
Cimbi-36	1.01 nM	1.7 nM	1255 nM	> 10.000 nM	1256 nM

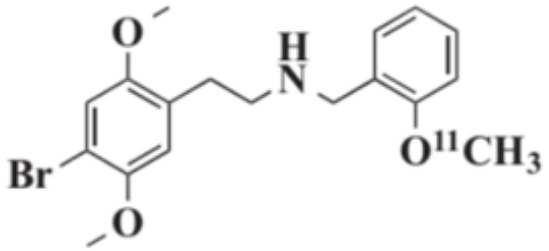
Guiard, *5-HT2A Receptors in the Central Nervous System* **2018**

Validation of [11C]-Cimbi-36

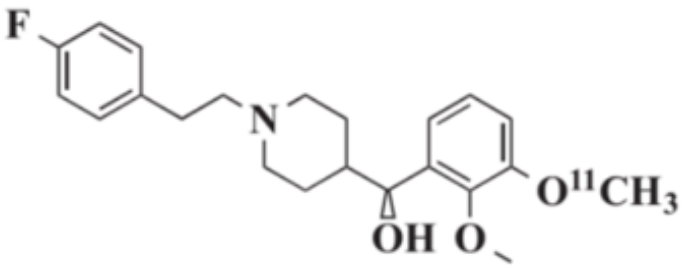
- [^{3}H]-Cimbi-36 displays substantial non-specific binding to both white/gray-matter tracts relative to [^{3}H]MDL 100907. Displacable-binding appears similar (10 uM block with ketanserin).

[^{3}H]MDL 100907 **[^{3}H]-Cimbi-36**





[^{11}C]Cimbi-36

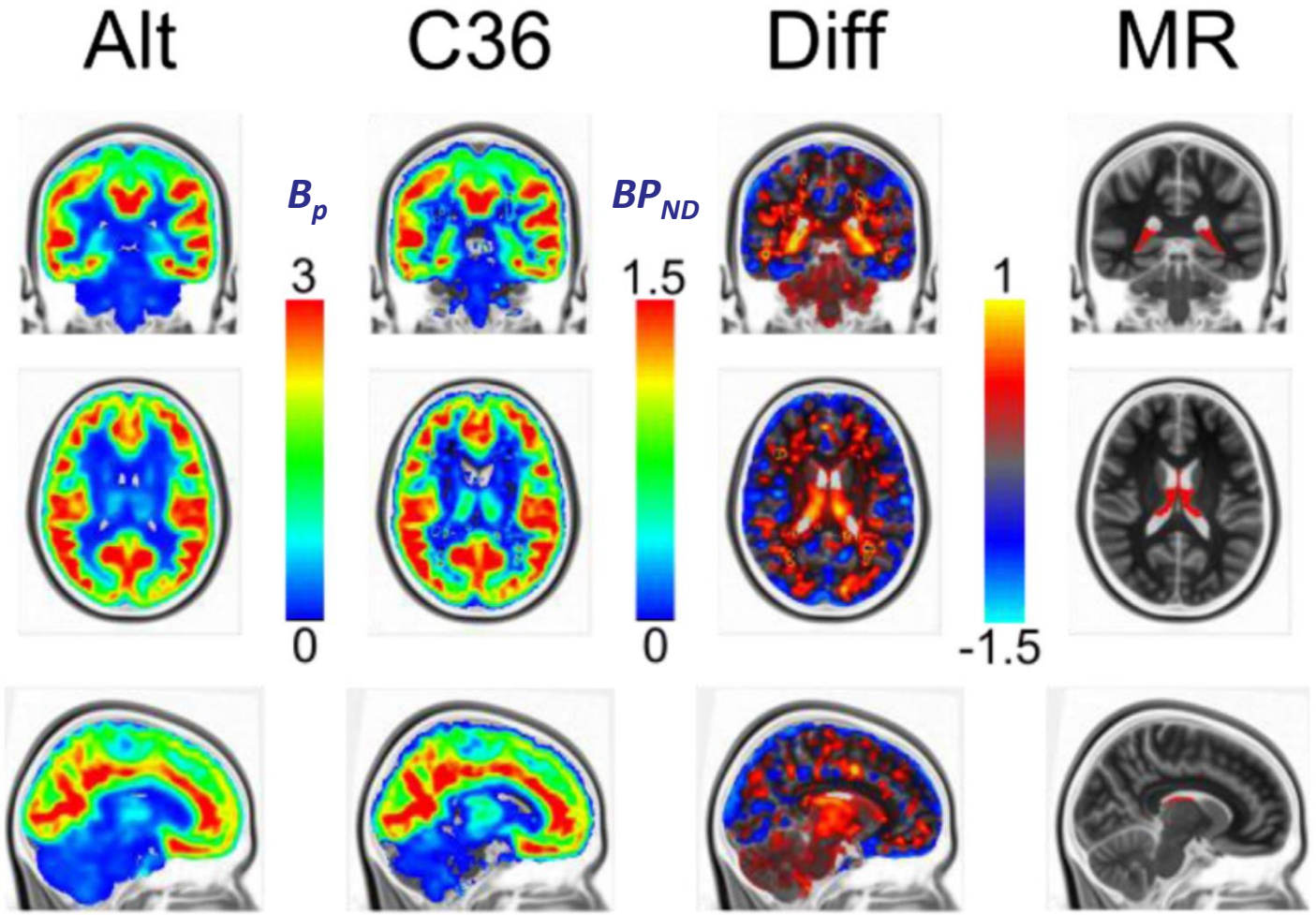


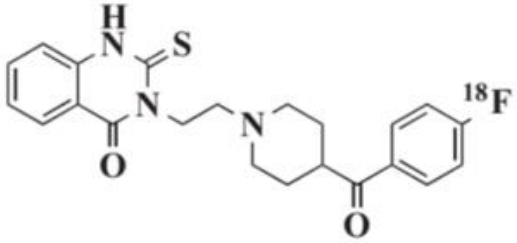
[^{11}C]MDL 100907

Finnema, S. J. et al. *NeuroImage* **2014** 84 342–353

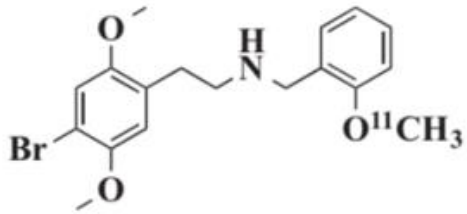
Validation of [11C]-Cimbi-36

- [^{11}C]-Cimbi-36 displays off-target binding to 5HT2c in humans, but appears comparable to established antagonist tracers (altanserin).





[^{18}F]altanserin



[^{11}C]Cimbi-36

Ettrup, A. et al. *NeuroImage* **2016**, 130 167–174

[11C]-Cimbi-36 RO study with Psilocybin: Study Design

- Subjects are dosed within the PET scanner (blinded to dose) and plasma drug levels area assessed every 20 mins over the duration of the scan; Lkert intensity scale is used during the scale: 0 = not intense 10 = intense.

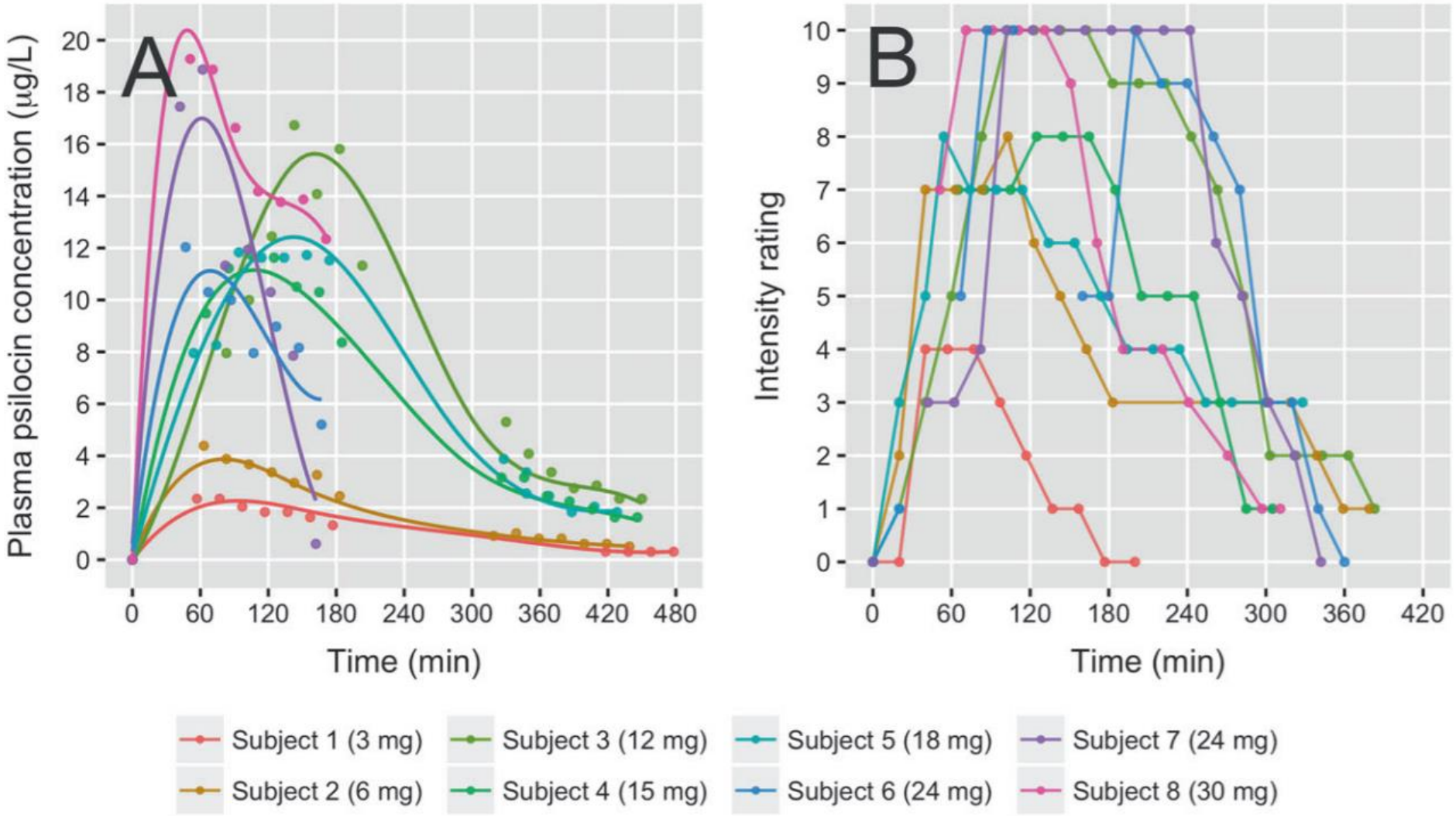
- Subjects are provided sedation if needed (not used within the study).

- Subjective questionnaires used at the end of the study include: 11-dimensional altered states of consciousness questionnaire, 30-item mystical experiences questionnaire and the ego-dissolution inventory
  - These are standard practice within the open-label trials described earlier.

Ettrup, A. et al. *NeuroImage* **2016**, 130 167–174

[11C]-Cimbi-36 RO study with Psilocybin: PK/PD relationship

- Plasma levels of Psilocin showed the expected dose-response relationship in terms of Likert Intensity scale.



Madsen, MK et al. *Neuropsychopharmacology* **2019** 44, 1328–1334

[11C]-Cimbi-36 RO study with Psilocybin: PK/PD relationship

- Compiled study data and potential limitations:

Table 1. Descriptive data related to psilocybin interventions and corresponding 5-HT2AR occupancy estimates

ID	Dose (mg)	Weight-adjusted dose (mg/kg)	C_{max} (µg/L)	Mean psilocin PET 1 (µg/L)	Mean psilocin PET 2 (µg/L)	Occupancy PET 1 (%)	Occupancy PET 2 (%)
Subject 1	3	0.05	2.3	1.9	<LOQ*	42.9	1.8
Subject 2	6	0.07	4.4	3.5	0.7	56.2	26.7
Subject 3	12	0.14	16.7	12.6	3.4	66.4	42.9
Subject 4	15	0.2	11.7	10.5	2.3	63.2	30.9
Subject 5	18	0.2	11.8	10.6	2.6	72.4	47.0
Subject 6	24	0.27	12.0	9.0	NA	60	NA
Subject 7	24	0.3	18.9	11.5	NA	66	NA
Subject 8	30	0.3	19.3	15.6	NA	65.2	NA

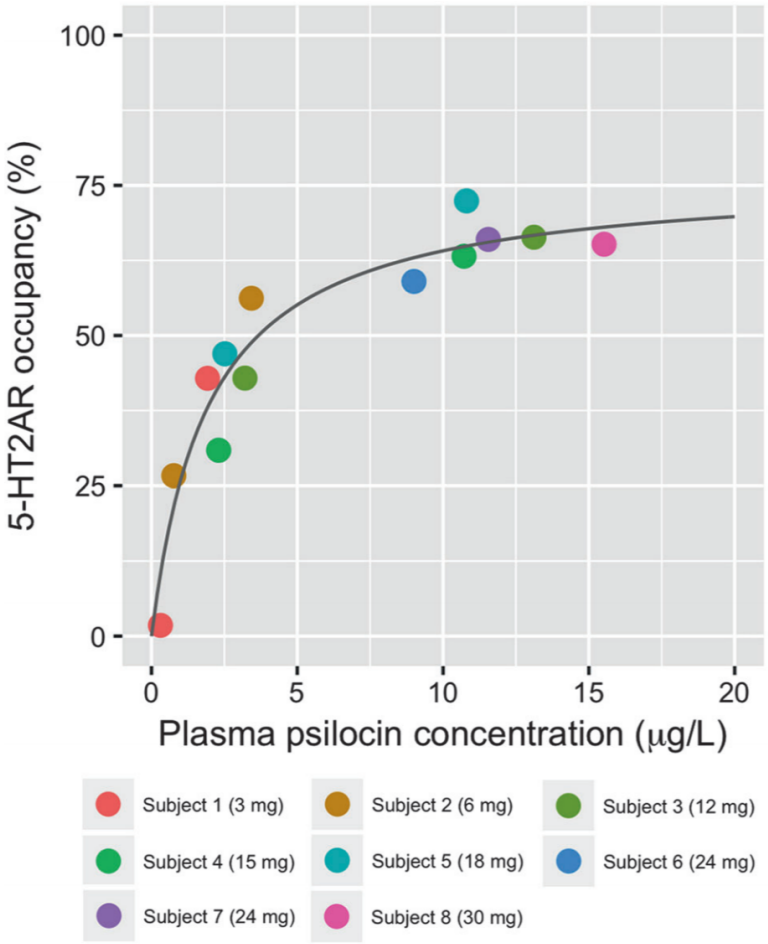
*Below level of quantification

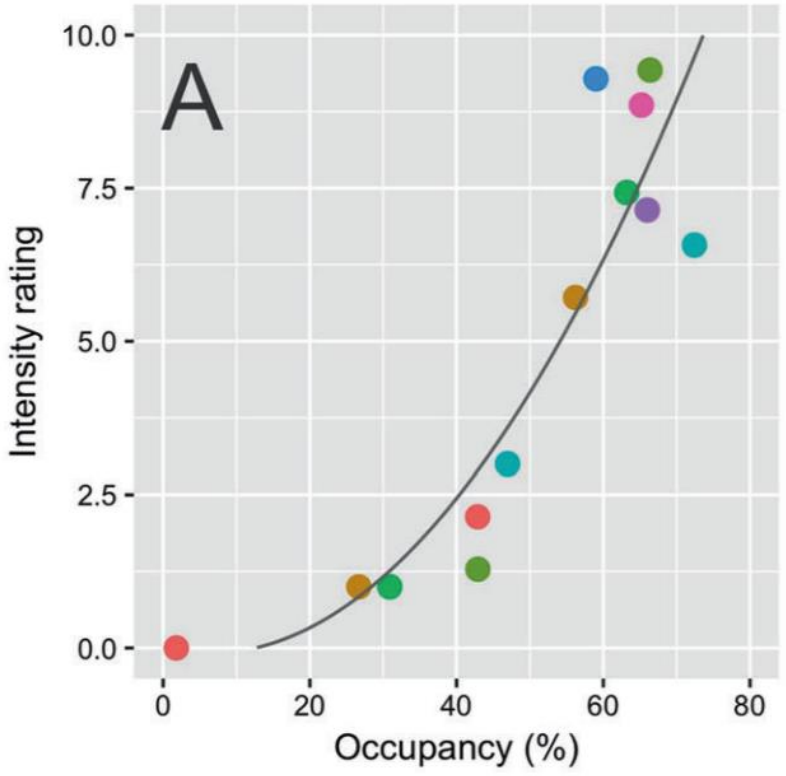
- Subject 1 returned to baseline within 60 mins of a low dose of Psilocybin; this is in contrast to numerous *in vitro* studies suggesting that 5HT2a receptor internalization occurs rapidly and is long lasting post dosing.

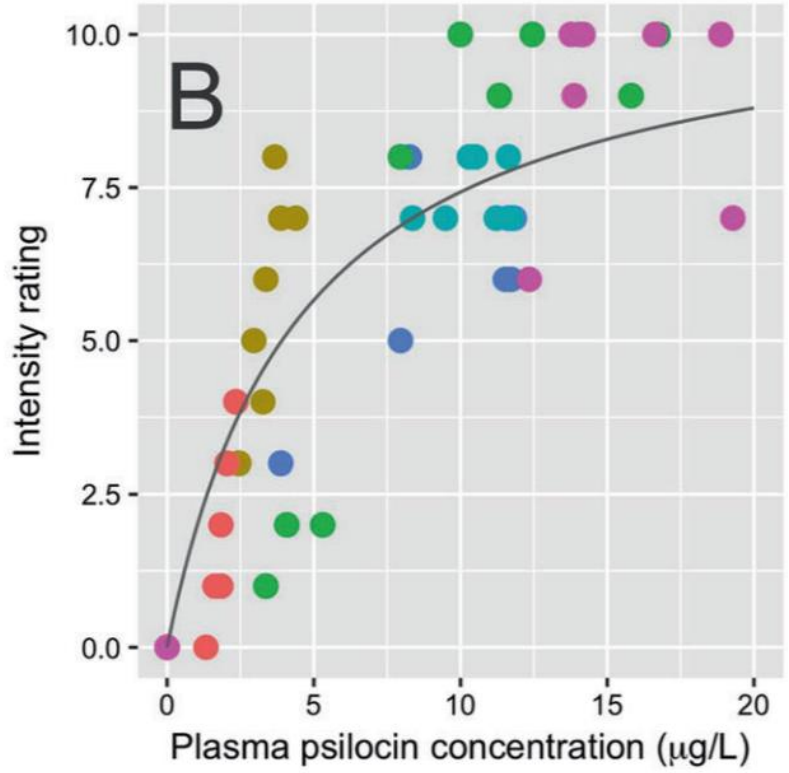
Madsen, MK et al. *Neuropsychopharmacology* **2019** 44, 1328–1334

[11C]-Cimbi-36 RO study with Psilocybin: PK/RO, RO/PD, PD/PK relationships

- Observed relationships between PK, PD and RO:







- Occ_{50} = 1.95 ug/L or 10 nM of psilocin (comparable to Ki values against [^{125}I]DOI in rat cortex; 6 nM or 25 nM).

Madsen, MK et al. *Neuropsychopharmacology* **2019** 44, 1328–1334

A Brief History of Psilocybin: From Isolation to Modern Clinical Trials

- Two phase 2/3 studies have been initiated by COMPASS and Usona in treatment resistant depression and major depressive disorder, respectively.
  - Both trials use distinct dosing paradigms (0.1 – 0.3 mpk or 25 mg; PO)
  - Both trials are expected to read out in 2020/2021

A Study of Psilocybin for Major Depressive Disorder (MDD)

The safety and scientific validity of this study is the responsibility of the study sponsor and investigators. Listing a study does not mean it has been evaluated by the U.S. Federal Government. Know the risks and potential benefits of clinical studies and talk to your health care provider before participating. Read our disclaimer for details.

ClinicalTrials.gov Identifier: NCT03866174

Recruitment Status : Recruiting
First Posted : March 7, 2019
Last Update Posted : April 22, 2020

See **Contacts and Locations**

Sponsor:
Usona Institute

The Safety and Efficacy of Psilocybin in Participants With Treatment Resistant Depression (P-TRD)

The safety and scientific validity of this study is the responsibility of the study sponsor and investigators. Listing a study does not mean it has been evaluated by the U.S. Federal Government. Know the risks and potential benefits of clinical studies and talk to your health care provider before participating. Read our disclaimer for details.

ClinicalTrials.gov Identifier: NCT03775200

Recruitment Status : Recruiting
First Posted : December 13, 2018
Last Update Posted : April 24, 2020

See **Contacts and Locations**

Sponsor:
COMPASS Pathways

A quick note on the tolerance effects of 5HT2a agonists: Naloxone prevents development of tolerance toward IV LSD in NHPs and rodents

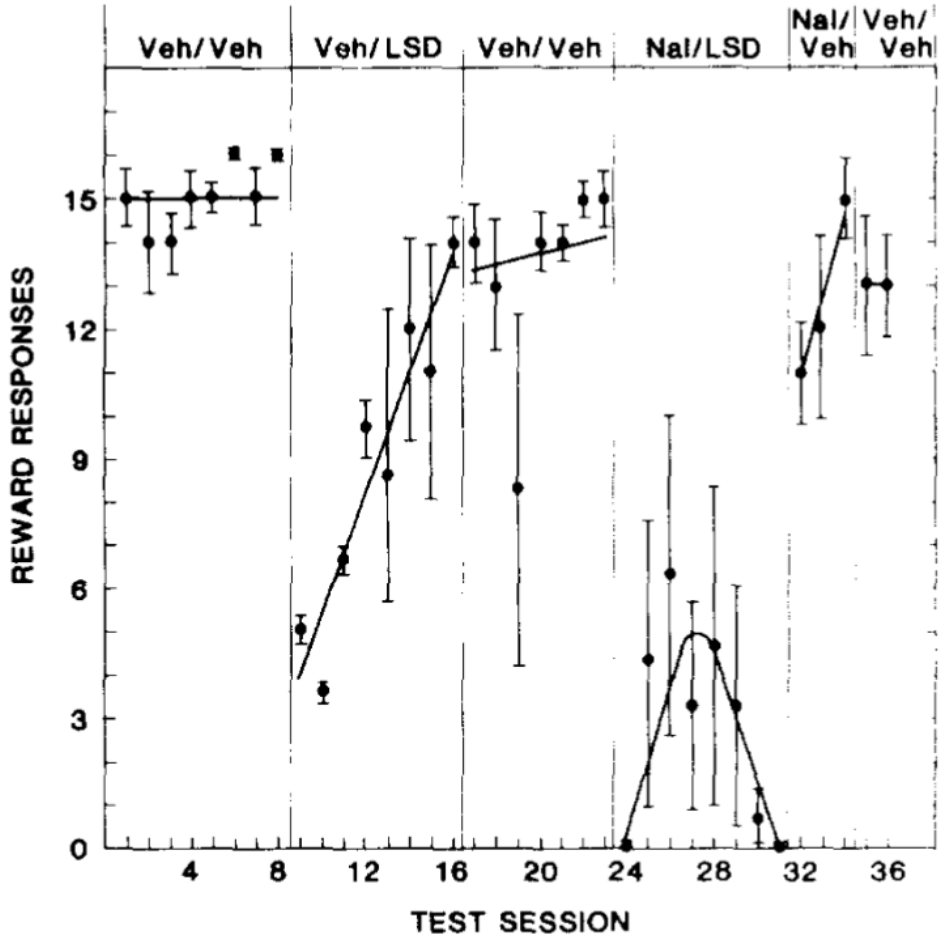
- Following up on rodent studies demonstrating that the tolerance of 5HT2a agonists (DMT, LSD, mescaline) could be modulated with opioid antagonists, Hadorn, Anistranski, and Connor report an unusual observation:

Table 1. Experimental design

Sessions	Injected 30 min prior to each test	Injected 15 min prior to each test
1–8	Vehicle	Vehicle
9–16	Vehicle	0.1 mg/kg LSD
17–23	Vehicle	Vehicle
24–31	1.0 mg/kg Naloxone	0.1 mg/kg LSD
32–34	1.0 mg/kg Naloxone	Vehicle
35–36	Vehicle	Vehicle

Each subject participated in two sessions per day.

Tolerance did not develop to these behavioral effects when naloxone was administered with LSD; rather they became more pronounced. Although the response rates for the task increased slightly after the first session, responding became progressively depressed in subsequent sessions. The naloxone–LSD regimen was discontinued when it became apparent that the animals were unable to respond and when concern developed about their well-being.



Hadorn, DC et al. *Neuropharmacology 1984, 23, 297-1300*