The relationship between Psilocybin/psilocin plasma levels and receptor occupancy: PET imaging with 11C-Cimbi-36
“It's tough to make predictions, especially about the future.”

-Yogi Berra
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.

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).

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

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

![Biosynthesis of Psilocybin](image)

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.

Psilocin displays “polypharmacology,” complicating mechanism-of-action studies.

<table>
<thead>
<tr>
<th>binding site</th>
<th>$K_i$ (nM)</th>
<th>binding site</th>
<th>$K_i$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SERT</td>
<td>3801</td>
<td>$\alpha_{1A}$</td>
<td>$&gt;10000$</td>
</tr>
<tr>
<td>5-HT$_{1A}$</td>
<td>567.4</td>
<td>$\alpha_{1B}$</td>
<td>$&gt;10000$</td>
</tr>
<tr>
<td>5-HT$_{1B}$</td>
<td>219.6</td>
<td>$\alpha_{2A}$</td>
<td>1379</td>
</tr>
<tr>
<td>5-HT$_{1D}$</td>
<td>36.4</td>
<td>$\alpha_{2B}$</td>
<td>1894</td>
</tr>
<tr>
<td>5-HT$_{2A}$</td>
<td>107.2, 25</td>
<td>$\alpha_{2C}$</td>
<td>$&gt;10000$</td>
</tr>
<tr>
<td>5-HT$_{2B}$</td>
<td>4.6</td>
<td>$\beta_1$</td>
<td>$&gt;10000$</td>
</tr>
<tr>
<td>5-HT$_{2C}$</td>
<td>97.3</td>
<td>$D_1$</td>
<td>$&gt;10000$</td>
</tr>
<tr>
<td>5-HT$_{3}$</td>
<td>$&gt;10000$</td>
<td>$D_2$</td>
<td>$&gt;10000$</td>
</tr>
<tr>
<td>5-HT$_{5}$</td>
<td>83.7</td>
<td>$D_3$</td>
<td>2645</td>
</tr>
<tr>
<td>5-HT$_{6}$</td>
<td>57.0</td>
<td>$D_4$</td>
<td>$&gt;10000$</td>
</tr>
<tr>
<td>5-HT$_{7}$</td>
<td>3.5</td>
<td>$D_5$</td>
<td>$&gt;10000$</td>
</tr>
<tr>
<td>$H_1$</td>
<td>304.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

Ketanserin
(selective 5HT2a antagonist)

5HT2a Ki = 2 nM
5HT2c Ki = 130 nM

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


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 Response
(60 patients)

2006
Psilocybin
0.1, 0.2, 0.3 mpk PO
OCD
(9 patients)

2011
Psilocybin
0.2 mpk PO
Existential Distress
(12 patients)

2014
Psilocybin
0.28, 0.42 mpk PO
Smoking Cessation
(15 patients)

2015
Psilocybin
0.3, 0.4 mpk PO
Alcohol Use Disorder
(10 patients)

2016
Psilocybin
0.3 mpk PO
Existential Distress
(29 patients)

2016
Psilocybin
0.28, 0.42 mpk PO
Existential Distress
(51 patients)

2016
Psilocybin
10, 20 mg PO
Treatment Resistance Depression

= double-blind, randomized, placebo-controlled

= open-label with or w/out placebo
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.
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.

\[
\begin{align*}
\text{Brain} & \quad \text{Blood} \\
[\text{drug}] & \quad [\text{drug}] \\
\text{CSF-brain barrier} & \quad \text{Blood-brain barrier} \\
\text{CSF-blood barrier} & \quad \text{CSF} \\
\end{align*}
\]

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.
Following peripheral administration (PO, IV, etc), a small molecule can distribute throughout the body. The unbound brain:plasma (K_{pu,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
- $C_{\text{max}} = 500 \text{ nM (plasma)}$
- $T_{\text{max}} = 1 \text{ hr}$
- $f_{\text{plasma}} = 0.5$
- $f_{\text{brain}} = 0.25$
- $K_D = 250 \text{ nM}$

Receptor occupancy = 50%
Following peripheral administration (PO, IV, etc), a small molecule can distribute throughout the body. The unbound brain:plasma \((K_{p_{u,u}})\) 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

\(K_{p_{u,u}} = < 0.1\)

Receptor occupancy = <5%
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 $K_{p_{u,u}}$ from pre-clinical species a risky proposition.

### TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>Mouse (fmol/µg)</th>
<th>Rat (fmol/µg)</th>
<th>NHP (fmol/µg)</th>
<th>Human (fmol/µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDR1</td>
<td>14.1</td>
<td>19.1</td>
<td>4.71</td>
<td>6.06</td>
</tr>
<tr>
<td>BCRP</td>
<td>4.41</td>
<td>4.95</td>
<td>14.2</td>
<td>8.14</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Animal</th>
<th>Proteomics</th>
<th>Parameter Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$f_{u,b}$</td>
<td>$f_{u,b,cor}$</td>
</tr>
<tr>
<td>Mouse</td>
<td>72%</td>
<td>77%</td>
</tr>
<tr>
<td>Rat</td>
<td>65%</td>
<td>72%</td>
</tr>
<tr>
<td>NHP</td>
<td>93%</td>
<td>73%</td>
</tr>
</tbody>
</table>

$f_{u,b}$, fraction unbound in brain; $f_{u,b,cor}$, corrected fraction unbound in brain; NHP, nonhuman primate; RAF, relative activity factor; REF, relative expression factor.
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 $^{11}\text{C}]\text{MK-4232}$ enabled the direct assessment of CGRP-R occupancy, confirming that central target engagement was not required for anti-migraine effects.

Telcagepant

$K_i = 0.77 \text{ nM}$

$P\text{-gp} = 24$

MK-3207

$K_i = 0.021 \text{ nM}$

$P\text{-gp} = 25$

$^{11}\text{C}]\text{MK-4232}$

$K_i = 0.039 \text{ nM}$

$P\text{-gp} = 1.7$

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.** [H]MK-4232 in vitro autoradiography of rhesus monkey brain slices.

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

**PET Receptor Occupancy Studies in CNS Drug Discovery: Basic Concepts**

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


- **MK-4232** $K_i = 0.039 \text{ nM (non-pgp substrate)}$

- **MK-3207** $K_i = 0.021$ Telc. $K_i = 0.77 \text{ nM (pgp substrates)}$

- **High Occupancy >90%**

- **Baseline (low occupancy <5%)**
**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

<table>
<thead>
<tr>
<th>Subject</th>
<th>Telcagepant Dose</th>
<th>Telcagepant Plasma Concentration</th>
<th>CGRP-R Occupancy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg</td>
<td>µM</td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>1120</td>
<td>16.3 ± 1.84</td>
<td>43</td>
</tr>
<tr>
<td>7</td>
<td>1120</td>
<td>20.2 ± 3.93</td>
<td>48</td>
</tr>
<tr>
<td>8</td>
<td>1120</td>
<td>22.2 ± 4.43</td>
<td>58</td>
</tr>
<tr>
<td>9</td>
<td>140</td>
<td>0.254 ± 0.140</td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td>140</td>
<td>0.859 ± 0.305</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>140</td>
<td>0.424 ± 0.187</td>
<td>4</td>
</tr>
</tbody>
</table>

A Brief History of Molecular Imaging at the 5HT2a receptor

Molecular Imaging at the 5HT2a receptor:

- 1950: The discovery of serotonin
- 1979: Radioligand binding studies with [3H]LSD and [3H]spiperone showed that 5-HT₁ and 5-HT₂ receptors exist
- 1983: [11C]NMSP was the first PET tracer that could image the D₂ and 5-HT₆ receptor systems
- 1995: [18F]altanserin was the first truly 5-HT₂A selective PET tracer
- 1996: [11C]MDL 100907 showed an improved selectivity profile for the 5-HT₂A receptor system, but unfavourable tracer kinetics
- 2011: [11C]Cimbi-36, the first agonist PET tracer for the 5-HT₂A receptor
- 2018: (R)-[18F]MH.MZ, combined the favourable selectivity profile of MDL 100907 with the superior imaging properties of fluoride-18

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

PET imaging at the 5HT2a receptor

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

![Chemical structures of probes](image)

<table>
<thead>
<tr>
<th></th>
<th>5-HT2A</th>
<th>5-HT2C</th>
<th>5-HT1A</th>
<th>D2</th>
<th>α1</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDL 100907</td>
<td>0.36 nM</td>
<td>107 nM</td>
<td>&gt; 10.000 nM</td>
<td>2250 nM</td>
<td>128 nM</td>
</tr>
<tr>
<td>(R)-MH.MZ</td>
<td>0.72 nM</td>
<td>53 nM</td>
<td>&gt; 10.000 nM</td>
<td>2686 nM</td>
<td>335 nM</td>
</tr>
<tr>
<td>altanserin</td>
<td>0.13 nM</td>
<td>6 nM</td>
<td>1570 nM</td>
<td>62 nM</td>
<td>4.55 nM</td>
</tr>
<tr>
<td>Cimbi-36</td>
<td>1.01 nM</td>
<td>1.7 nM</td>
<td>1255 nM</td>
<td>&gt; 10.000 nM</td>
<td>1256 nM</td>
</tr>
</tbody>
</table>
Validation of [11C]-Cimbi-36

[3H]-Cimbi-36 displays substantial non-specific binding to both white/gray-matter tracts relative to [3H]MDL 100907. Displacable-binding appears similar (10 uM block with ketanserin).
Validation of [11C]-Cimbi-36

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

Subjects are dosed within the PET scanner (blinded to dose) and plasma drug levels are assessed every 20 mins over the duration of the scan; Likert 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.

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
Compiled study data and potential limitations:

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

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

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

*Below level of quantification

- Observed relationships between PK, PD and RO:

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

Madsen, MK et al. *Neuropsychopharmacology* 2019 44, 1328–1334
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 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>
<thead>
<tr>
<th>Sessions</th>
<th>Injected 30 min prior to each test</th>
<th>Injected 15 min prior to each test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-8</td>
<td>Vehicle</td>
<td>Vehicle</td>
</tr>
<tr>
<td>9-16</td>
<td>Vehicle</td>
<td>0.1 mg/kg LSD</td>
</tr>
<tr>
<td>17-23</td>
<td>Vehicle</td>
<td>Vehicle</td>
</tr>
<tr>
<td>24-31</td>
<td>1.0 mg/kg Naloxone</td>
<td>0.1 mg/kg LSD</td>
</tr>
<tr>
<td>32-34</td>
<td>1.0 mg/kg Naloxone</td>
<td>Vehicle</td>
</tr>
<tr>
<td>35-36</td>
<td>Vehicle</td>
<td>Vehicle</td>
</tr>
</tbody>
</table>

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