Pharmacokinetic modeling of PET neuroimaging data

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In vitro receptor binding concepts

- $B_{\text{max}} = \text{concentration of receptor sites}$
- $K_D = \text{dissociation contrast}$
  - (conversely, $1/K_D = \text{affinity of each receptor}$)
- $BP_F = B_{\text{max}} / K_D = \text{binding potential}$
The Law of Mass Action

"The rate of association is proportionate to the concentrations of the reactants, and the rate of dissociation is proportionate to the concentration of the complex."

\[ L + R \overset{k_{on}}{\rightleftharpoons} LR \overset{k_{off}}{\rightarrow} \]

- \( L \) = ligand
- \( R \) = receptor
- \( LR \) = ligand-receptor complex
- \( k_{on} \) = the rate constant of association
  - = bimolecular association rate (nM\(^{-1}\)min\(^{-1}\))
- \( k_{off} \) = the rate constant of dissociation (min\(^{-1}\))
The Law of Mass Action

Thus, \([LR]\) will increase in proportion to the product \([L][R]\) and decrease in proportion to \([LR]\):

\[
\frac{d[LR]}{dt} = k_{on} [L][R] - k_{off} [LR]
\]
Dynamic equilibrium

- At equilibrium, the rate of association equals the rate of dissociation:

\[
\frac{d[LR]}{dt} = 0, \text{ thus } k_{\text{on}}[L][R] = k_{\text{off}}[LR]
\]

rearrangement gives:

\[
\frac{k_{\text{off}}}{k_{\text{on}}} = \frac{[L][R]}{[LR]} = K_D
\]

Dissociation constant, units of concentration (nM)
"Michaelis-Menten" equation for receptor binding

- **Redefine:**
  \[ B = [LR] = \text{concentration of bound ligand} \]
  \[ F = [L] = \text{concentration of free (unbound) ligand} \]

- **Total concentration of receptors:**
  \[ B_{\text{max}} = [LR] + [R] \]

- **Concentration of available receptors:**
  \[ B_{\text{max}}' = B_{\text{max}} - B = [R] \]
"Michaelis-Menten" equation for receptor binding

Thus:

\[
K_D = \frac{k_{\text{off}}}{k_{\text{on}}} = \frac{[L][R]}{[LR]} = \frac{FB_{\text{max}}'}{B} = \frac{F(B_{\text{max}} - B)}{B}
\]
"Michaelis-Menten" equation for receptor binding

Solving for B:

\[ B = \frac{B_{\text{max}} F}{K_D + F} \]

The "Michaelis-Menten" relationship
Saturation binding curve  

\[ B = \frac{B_{\text{max}} F}{K_D + F} \]

- **\( B_{\text{max}} \)** is 100 nM.
- **\( B \)** will asymptotically approach **\( B_{\text{max}} \)** in theory, when all receptors are saturated, **\( B = B_{\text{max}} \)**.
- **\( K_D \)** is the concentration at which half the receptors are saturated: when **\( B = B_{\text{max}} / 2 \)**, **\( F = K_D \)**.

Image courtesy of Robert B. Innis (NIMH, USA)
Saturation binding curve

- Slope of the saturation binding curve:

\[
\frac{B}{F} = \frac{B_{\text{max}}}{K_D + F}
\]
PET: tracer doses

• In PET, minuscule amounts of the radiotracer are injected ("tracer" dose)
• Only <1% of the receptors are occupied (ideally)
• No pharmacological effects expected
• Specific activity (SA, MBq/nmol): amount of labeled molecules relative to unlabeled ("cold", "carrier") molecules
  – High SA: tracer dose, <1% occupancy
  – Low SA: significant occupancy at receptors!
PET: tracer doses

• Thus, $F<<K_D$ (the latter being the concentration at which 50 % of the receptors are occupied), and:

$$\frac{B}{F} = \frac{B_{\text{max}}}{K_D} = B_{\text{max}} \times \text{Affinity} = BP$$
Saturation binding curve

BP is the initial slope of the saturation binding curve - At tracer doses, the slope (BP) is constant and the association of Bound and Free nearly linear

slope = $B_{\text{max}}/K_d = \text{BP} = 50$

Image courtesy of Robert B. Innis (NIMH, USA)
Scatchard linearization

• Rearrangement of the "Michaelis-Menten" equation gives:

\[
\frac{B}{F} = \left( \frac{-1}{K_D} \right) B + \frac{B_{max}}{K_D}
\]

Slope = \(-1/K_D\)  \hspace{1cm} Y-intercept = \(B_{max}/K_D\)
Scatchard linearization

With PET tracer doses, we operate in this portion of the Scatchard plot.

\[ \frac{B_{\text{max}}}{K_d} = \text{BP} = 50 \]

slope = \(-\frac{1}{K_d} = -0.5\)

\[ B_{\text{max}} = 100 \]
Major differences between *in vitro* measurements and *in vivo* PET

- *In vivo* PET: usually, tracer doses are used ($F \ll K_D$)
- Thus, receptors are not occupied at all $\rightarrow B_{\text{max}}$ or $K_D$ cannot be measured separately, only their ratio ($BP_F$)!
- *In vitro*, multiple levels of saturation is used to describe $B_{\text{max}}$ and $K_D$
- *In vivo* PET: regional blood flow, extraction, binding to plasma proteins, non-specific binding, multiple populations of specific binding sites, internal milieu (pH, ion concentrations etc), radioactive metabolites of the radiotracer, endogenous neurotransmitters, factors related to PET instrumentation…
Interpretation of *in vivo* BP differences

• From a pharmacological point of view, if $BP_F$ differs between individuals, what’s different?

- $B_{\text{max}}$: different individuals have different concentrations of receptors

- $K_D$: property of a single receptor: eg. conformational changes in the receptor protein structure may lead to differences in $K_D$
Receptor occupancy

Depleted ← Baseline ← Stimulated

γ D2 receptor □ [C-11]raclopride ▼ Dopamine

Competitive inhibition with PET

- Endogenous neurotransmitters or exogenous substances compete with the radioligand in binding to receptors
- Competitive inhibition can be studied with PET using two tracer dose scans: one in baseline, and another after pharmacological challenge
- Changes in BP are considered to reflect changes in the synaptic concentrations of endogenous neurotransmitters
Competitive inhibition with PET

• But… what changes \textit{in vivo} $BP_F$ in competitive inhibition?
  
  – $B_{\text{max}}$: the total concentration of receptor \textit{cannot} change, otherwise not competitive inhibition!
  
  – $K_D$: the affinity of each receptor \textit{cannot} change in competitive inhibition!

• Introducing a new term: apparent affinity

\[
\frac{1}{K_D^{\text{app}}} = \frac{1}{K_D \left(1 + \sum \frac{F_i}{K_D} \right)}
\]
Pharmacological interpretation of $BP_F$ \textit{in vivo}

$$BP = \frac{B_{\text{max}}}{K_{D}^{\text{app}}} = \frac{B_{\text{max}}}{K_D \left( 1 + \sum \frac{F_i}{K_{D_i}} \right)}$$

$K_D = \text{equilibrium dissociation constant of the tracer}$

$F_i = \text{concentration of } i \text{ competing substances}$

$K_{D_i} = \text{equilibrium dissociation constant of } i \text{ competing substances}$
Occupancy

• For the measurement of occupancy of endogenous or exogenous ligands using two PET scans with tracer doses:

\[
\text{Occupancy (\% ) } = \frac{BP_{\text{BEFORE}} - BP_{\text{AFTER}}}{BP_{\text{BEFORE}}} \times (100\%)
\]
Scatchard analysis *in vivo* for the differentiation of $B_{\text{max}}$ and $K_D$

- Multiple PET scans are needed with decreasing specific activities
  - Thus, gradually increasing the amount of unlabeled ("cold") radioligand to yield significant occupancy at the receptors
- From multiple observations, pairs of $B$ and $B/F$ are calculated and plotted in the Scatchard plot
  - $B$ can be measured at equilibrium as $C_B(t)/SA$, where $C_B(t)=C_T(t)-C_{\text{REF}}(t)$
  - $B/F$ can be measured as $C_B/C_{\text{REF}}$
Scatchard analysis *in vivo* for the differentiation of $B_{\text{max}}$ and $K_D$

- High SA, negligible occupancy
- Gradually decreasing SA, increasing occupancy

**Equation**

$$\frac{B_{\text{max}}}{K_d} = \text{BP} = 50$$

**Slope**

$$-\frac{1}{K_d} = -0.5$$

**Parameters**

- $B_{\text{max}} = 100$

Image courtesy of Robert B. Innis (NIMH, USA)
Confounding factors and complications

• Properties of the radioligand
  – Target receptor population (affinity states etc.)
  – Physiological receptor variants
  – Is it comparable to the endogenous ligand?
• Receptor trafficking
  – Agonist-induced receptor internalization
  – How does is affect $B_{max}$?
  – Do PET radioligands bind to internalized receptors? How?
• Non-competitive inhibition, changes in receptor conformation
Full compartmental model

Practically, too many parameters to achieve reliable fits...
Full compartmental model

- $C_P = \text{radioactivity concentration in arterial plasma}$
- $C_F = \text{radioactivity concentration of free radioligand in tissue}$
- $C_B = \text{radioactivity concentration of specifically bound radioligand}$
- $C_{NS} = \text{radioactivity concentration of non-specifically bound radioligand}$
- $K_1 = \text{rate constant for transit between plasma and tissue (ml tissue)/(ml plasma)/min}$
- $k_2 = \text{rate constant for transit between tissue and plasma (min}^{-1})$
- $k_3, k_4 = \text{rate constants for transit between free and specifically bound compartments and vice versa (min}^{-1})$
- $k_5, k_6 = \text{rate constants for transit between free and non-specifically bound compartments and vice versa (min}^{-1})$
Assumption in all compartmental models

- Only free radioligand in arterial plasma in considered to pass the blood-brain barrier
- Free radioligand in plasma = not bound to proteins
- The fraction of total plasma radioactivity originating from free radioligand = $f_P$
Standard 3-compartmental model

\[ C_{PET} = (1 - V_b)C_T + V_b C_{wb}; \quad C_T = C_{F+NS} + C_B \]
Assumptions in the 3-compartmental model

- Free and non-specifically bound compartments are assumed to be at equilibrium rapidly.
- Thus, these are treated as a single compartment.
- The fraction of radioactivity in this combined compartment originating from free radioligand = $f_{ND}$
Volume of distribution ($V_T$)

- The ratio of radioactivity concentration in a compartment and in plasma, thus:

$$V_j = \frac{C_j}{f_P C_P}$$

$V_j$ = the distribution volume of the $j$th compartment  
$C_j$ = radioactivity concentration in the $j$th compartment  
$f_P$ = plasma "free fraction"  
$C_P$ = radioactivity concentration in arterial plasma
Derivation of $V_T$ from rate constants: total $V_T$ for 2-compartmental model

\[
\frac{dC_T}{dt} = K_1 C_P - k_2 C_T
\]

\[
C_T = C_{F+NS} + C_B
\]
Derivation of $V_T$ from rate constants: total $V_T$ for 2-compartmental model

- At equilibrium, no net transfer between plasma and tissue, thus:

$$\frac{dC_T}{dt} = 0 ;$$

$$K_1 C_P = k_2 C_T$$

and

$$V_T = \frac{C_T}{C_P} = \frac{K_1}{k_2}$$
Derivation of $V_T$ from rate constants: total $V_T$ for 3-compartmental model

\[ C_T = C_{F+NS} + C_B \]

\[ \frac{dC_{F+NS}}{dt} = K_1 C_P - k_2 C_{F+NS} - k_3 C_{F+NS} + k_4 C_B \]

\[ \frac{dC_B}{dt} = k_3 C_{F+NS} - k_4 C_B \]
Derivation of $V_T$ from rate constants: total $V_T$ for 3-compartamental model

- At equilibrium:

$$\frac{dC_B}{dt} = 0 \Rightarrow k_3 C_{F+NS} = k_4 C_B ; \quad C_B = \frac{k_3}{k_4} C_{F+NS}$$

Thus

$$V_T = \frac{C_T}{C_p} = \frac{C_{F+NS} + C_B}{C_p} = \left(1 + \frac{k_3}{k_4}\right) \frac{C_{F+NS}}{C_p}$$
Derivation of $V_T$ from rate constants: total $V_T$ for 3-compartmental model

• At equilibrium:

$$C_{F+NS} = \frac{K_1}{k_2} C_P$$

thus:

$$V_T = \left( \frac{K_1}{k_2} \right) \left( 1 + \frac{k_3}{k_4} \right)$$
How do rate constants relate to pharmacological binding parameters?

\[ k_3 = k_{on} f_{ND} \left( B_{max} - \frac{C_B(t)}{SA} \right) \]

\[ k_4 = k_{off} \]
How do rate constants relate to pharmacological binding parameters?

- At tracer doses, $SA >> C_P(t)$ (that is, negligible occupancy by the radiotracer), and $k_3$ formula reduces to:

$$k_3 = k_{on} f_{ND} B_{max}$$

Since

$$\frac{k_{offs}}{k_{ons}} = K_D,$$

then

$$\frac{k_3}{k_4} = \frac{f_{ND} B_{max}}{K_D} = BP_{ND}$$
Distribution Volume ($V_T$)

$V_T$ equals uptake in brain relative to how much activity is delivered in arterial plasma.

$$V_T = \frac{\text{Area Brain Curve}}{\text{Area Plasma Curve}}$$

$$V_T = \frac{16}{2} = 8$$
Distribution Volume ($V_T$)

$V_T$ equals uptake in brain relative to how much activity is delivered in arterial plasma

\[
V_T = \frac{\text{Area Brain Curve}}{\text{Area Plasma Curve}}
\]

\[
V_T = \frac{?}{2} = ?
\]
Two inverse agonist radioligands for cannabinoid CB₁ receptors

$[^{11}\text{C}]\text{MePPEP}$

$T_{1/2} = 20 \text{ min}$
Two inverse agonist radioligands for cannabinoid $\text{CB}_1$ receptors

$[^{11}\text{C}]\text{MePPEP}$
## Comparison of $^{11}$C-MePPEP and $^{18}$F-FMPEP-$d_2$

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Intersubject variability $(n = 17)$  
Retest variability $(n = 9)$

Retest variability $(n = 8)$

Two inverse agonist radioligands for cannabinoid CB₁ receptors

\[ ^{11}\text{C}\text{MePPEP} \]

\[ ^{18}\text{F}\text{FMPEP}-d_2 \]

\[ T_{1/2} = 20 \text{ min} \]

\[ T_{1/2} = 110 \text{ min} \]
Two inverse agonist radioligands for cannabinoid CB₁ receptors

$[^{11}\text{C}]\text{MePPEP}$

$[^{18}\text{F}]\text{FMPEP-d}_2$
Comparison of $^{11}$C-MePPEP and $^{18}$F-FMPEP-d$_2$

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Intersubject variability $(n = 17)$ $(n = 9)$
Retest variability $(n = 8)$ $(n = 8)$

Standard 3-compartmental model

\[ C_P \quad \xrightarrow{K_1} \quad C_{F+NS} \quad \xrightarrow{k_3} \quad C_B \]

\[ C_{ND}, V_{ND} \quad \text{and} \quad C_S, V_S \]
## Nomenclature

<table>
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<tr>
<th>BP notation</th>
<th>Pharmacological interpretation</th>
<th>Kinetic interpretation</th>
<th>$V_T$ interpretation</th>
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<td>$\frac{B_{max}}{K_D}$</td>
<td>$\frac{K_1 k_3}{f_P k_2 k_4}$</td>
<td>$\frac{V_T - V_{ND}}{f_P}$</td>
<td>No</td>
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<td>$\frac{f_{ND} B_{max}}{K_D}$</td>
<td>$\frac{k_3}{k_4}$</td>
<td>$\frac{V_T}{V_{ND}} - 1$</td>
<td>No</td>
<td>Yes</td>
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Methods for estimating BP in vivo

• Direct method
  – From rate constants: complicated

• Indirect method
  – Calculation from $V_T$ values derived from target and reference regions using arterial plasma input: more robust
  – Calculation using reference region models: robust, arterial blood sampling not required
  – Caveat: critically dependent on the validity of the reference region to accurately estimate $V_{ND}$

\[
\frac{K_1k_3}{f_p k_2 k_4} = \frac{B_{\text{max}}}{K_D} = BP_F
\]

\[
\frac{k_3}{k_4} = \frac{f_{ND}B_{\text{max}}}{K_D} = BP_{\text{ND}}
\]

\[
V_T - V_{ND} = \frac{f_p B_{\text{max}}}{K_D} = BP_{P}
\]

\[
\frac{V_T}{V_{ND}} - 1 = \frac{f_{ND} B_{\text{max}}}{K_D} = BP_{\text{ND}}
\]
Reference region methods

\[ C_p \rightarrow K_1 \rightarrow C_{F+NS} \rightarrow k_3 \rightarrow C_B \]

\[ C_{REF} \]

Flow directions are indicated by arrows.
Reference region methods

- Estimation of the free and non-specific compartment \((C_{F+NS})\) from a reference region would obviate the need of arterial blood sampling
  - A major advantage in clinical studies!
- In a valid reference region, \(V_{ND}\) represents only free and non-specific radioligand – no specific binding to receptors
- Central assumption: free and non-specific binding is same between brain regions, i.e.:

\[
\frac{K_1}{k_2} = \frac{K'_1}{k'_2}
\]

Note that blood flow is not assumed to be equal across brain regions - only the ratio \(K_1/k_2\).
Reference region methods: indirect BP estimation from $V_T$ values

\[ V_T = \left( \frac{K_1}{k_2} \right) \left( 1 + \frac{k_3}{k_4} \right) \]

Measured from the ROI

\[ V_T = V_{REF} = \frac{K'_1}{k_2} \]

Measured from the reference region

Assumption:

\[ \frac{K_1}{k_2} = \frac{K'_1}{k_2} \]

Thus:

\[ \frac{V_T}{V_{REF}} - 1 = \frac{\left( \frac{K_1}{k_2} \right) \left( 1 + \frac{k_3}{k_4} \right)}{\left( \frac{K_1}{k_2} \right)} - 1 = \frac{k_3}{k_4} = \frac{f_{ND} B_{max}}{K_D} (BP_{ND}) \]
Reference region methods: indirect BP estimation from $V_T$ values

\[ V_T = \left( \frac{K_1}{k_2} \right) \left( 1 + \frac{k_3}{k_4} \right) \]

Measured from the ROI

\[ V_T = V_{REF} = \frac{K'_1}{k'_2} \]

Measured from the reference region

Assumption:

\[ \frac{K_1}{k_2} = \frac{K'_1}{k'_2} \]

Accordingly:

\[ V_T - V_{REF} = \left( \frac{K_1}{k_2} \right) \left( 1 + \frac{k_3}{k_4} \right) - \left( \frac{K_1}{k_2} \right) = \frac{K_1 k_3}{k_2 k_4} = \frac{f_p B_{\text{max}}}{K_D} \quad (BP_P) \]
Reference region methods: simplified reference tissue model (SRTM)

Further assumptions: bound and free+non-specific compartments reach equilibrium rapidly → they can be treated as a single compartment, $C_{F+NS+B}$

$$k_{2}^{app} = \frac{k_{2}}{1 + BP}$$

$$C_{T}(t) = R_{1}C_{REF}(t) + \left( k_{2} - \frac{R_{1}k_{2}}{1 + BP} \right)C_{REF}(t) \otimes e^{-\left(\frac{k_{2}t}{1 + BP_{ND}}\right)}$$

$C_{T}(t) = $ radioactivity concentration in the region of interest ($=C_{F+NS}+C_{B}$)

$C_{REF}(t) = $ radioactivity concentration in the reference region

$R_{1} = $ ratio of $K_{1}$ and $K_{1}'$

$BP_{ND} = $ binding potential
Scenario 1.

• Radioligand 1 has no reference region, you choose:

\[ \frac{V_T}{f_P} \quad V_T \quad BP_F \]
Scenario 2.

• Radioligand 2 may have different plasma protein binding ($f_P$) between subjects, difficult to measure… you choose:

$BP_{ND}$
Scenario 3.

- Radioligand 3 has a brain-penetrant radiometabolite, you choose:
Conclusions

• Nomenclature concerning the parameters estimates for specific binding may be confusing

• **Always** check what is really meant by ”BP”

• **Always** state explicitly in an article what you mean by ”BP”

• Keep in mind the limitation and vulnerabilities of each model

• Learn the model configurations and common formulas