

Tracer Kinetic Modeling

Introduction

A dynamic PET study starts with the injection of the radioligand and traces its distribution in the organism or a particular organ of interest over time. The PET data are divided into several time frames. A typical time activity curve is shown in figure 1 and shows the tracer accumulation of the D2/D3 receptor antagonist [^{11}C]raclopride in the mouse brain after intravenous bolus injection.

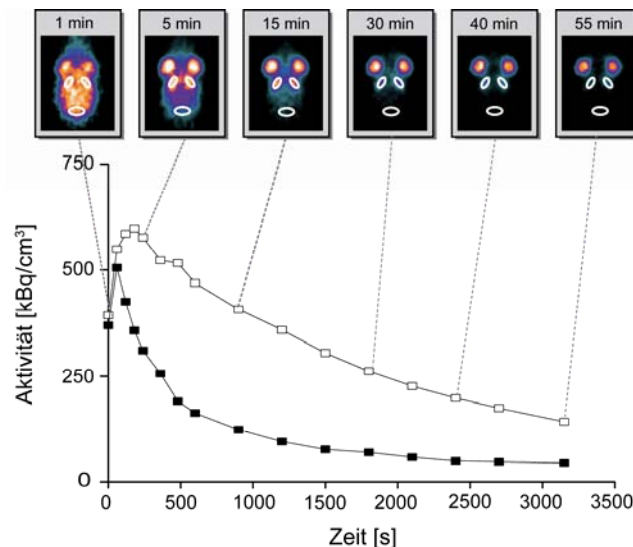


Figure 1: Activity distribution in the mouse brain after bolus injection of [^{11}C]raclopride. Coronal PET images (top) and time activity curves of the striatum (white quadrangle) and Cerebellum (black quadrangle) show the pharmacokinetic of the radioligand over time. Modified from Judenhofer et al. (2011).

Kompartiment Models

The activity distribution in tissue as measured by PET can be described by subdivision of the entire radioactivity into subsystems, so-called *compartments*. A compartment is not a physical volume, but rather a state of the radioligand (f.e. bound or free). There are three general assumptions that have to be met in order to use *compartment modeling* for the quantification of PET data (Leenders, 2011):

1. The physiological processes and molecular interactions are not influenced by the PET measurement (**tracer assumption**).
2. The physiological process and molecular interactions are in a constant state (steady state) during the PET experiment (**steady state assumption**).
3. The concentration in the compartment is homogeneous (**instantaneous mixing assumption**)

One of the most commonly used illustrations to describe the radioligand concentration is shown in figure 2. A region in the brain containing specific binding sites can be described by the following compartments: radioligand in plasma (C_P),

free in tissue (C_F), unspecifically bound (C_{NS}) and specifically bound (C_S). The total distribution in tissue results from $C_F + C_{NS} + C_S$. The transfer between the compartments is described by six first order rate constants K_1 to k_6 . Due to the large number of parameters (six) is this model very complex, hard to implement and error-prone. Under the assumption that the free and non-specifically bound radioligand exist in a fast equilibrium state compared to the specifically bound radioligand, i.e. k_5 and k_6 are very fast in relative comparison to the other rate constants, then C_F and C_{NS} can be combined in one compartment ($C_{NS} + C_F$). The 3-compartment-model is reduced to a 2-compartment-model with only four parameters. The rate constants K_1 and k_2 describe the transfer of the radioligand from blood to tissue and back, while k_3 and k_4 describe the association and dissociation of the radioligand to the specific receptor. If k_3 and k_4 are much faster in comparison to K_1 and k_2 , then a 2-compartment-model can be further reduced to a 1-compartment-model in receptor binding studies ($C_{NS} + C_S$). Simplified methods have the big advantage that they usually describe the behaviour of the radioligand in tissue far better, since they are less susceptible for errors due to the reduction of the fitting parameters. For the quantification of PET data, not only the knowledge of the radioligand distribution in tissue, but also the delivery into the tissue is needed. In general two types of compartment models can be distinguished: plasma input models and reference tissue models. Plasma input models are the gold standard method since they incorporate the radioligand distribution in blood plasma over time in the form of an arterial input function for the quantification of the PET data. Reference tissue models have been developed as an alternative method to the plasma-input models and have the big advantage, that they are non invasive and therefore particularly in small animals (rats and mice) easily applicable. They are based on the assumption that a reference region without specific binding sites exist from which an *indirect* input function can be generated. The reference region is described by a 1-compartment-model and assumes that the ratio of K_1/k_2 is equal in both tissues:

$$\frac{K_1}{k_2} = \frac{K_1'}{k_2'} \quad [1]$$

K_1' and k_2' describe the transfer of the radioligand from blood into the reference tissue and back.

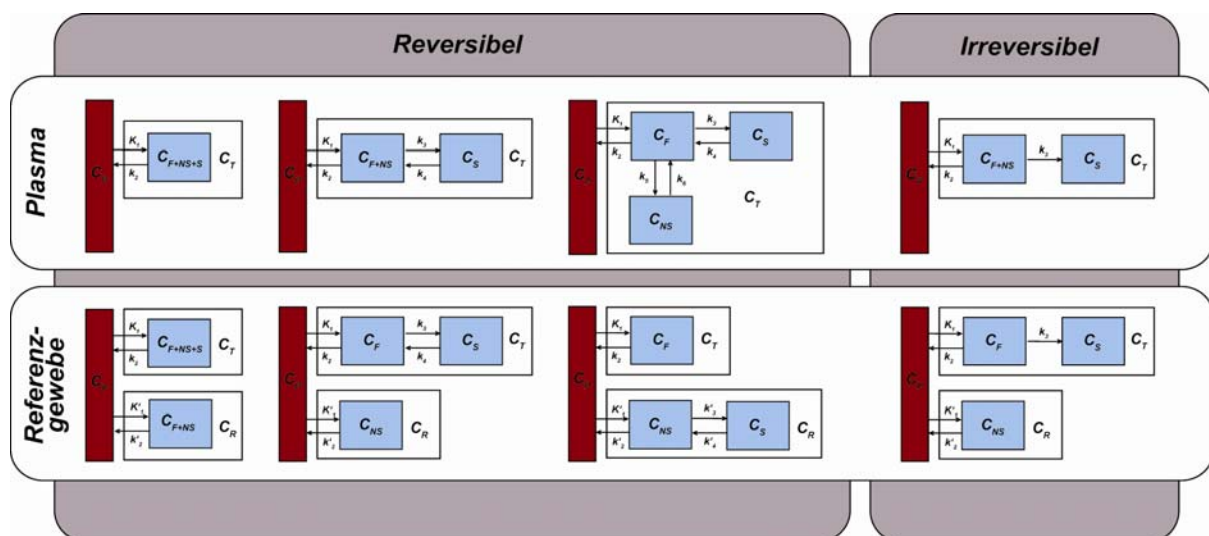


Figure 2: Frequently used compartment models in PET for reversible (left) and irreversible (right) ligands, with plasma input (top) and with reference tissue (bottom). Modified after Gunn et al. (2002).

Quantitative Parameters

Binding Potential. The binding potential is one of the most commonly used measures to describe the ligand-receptor-interaction and is derived from Michaelis-Menten-Kinetics:

The reversible binding of a ligand (L) to its receptor (R) to the ligand-receptor-complex (LR) can be described by the following equation:



k_{on} is the rate constant for the association to the receptor while k_{off} is the rate constant which describes the dissociation from the receptor. Under equilibrium conditions, the association and dissociation rate constant are equal.

$$k_{on}[L][R] = k_{off}[LR] \quad [3]$$

Under equilibrium conditions the ratio of the rate constants is constant and is named the equilibrium rate constant (K_d):

$$\frac{k_{off}}{k_{on}} = \frac{[L][R]}{[LR]} = K_d \quad [4]$$

In receptor binding studies the receptor density (B_{max}) is equal to entire receptor density, which is composed of free available receptor sites $[R]$ and with ligand bound receptor sites $[LR]$:

$$B_{max} = [LR] + [R] \quad [5]$$

Redefinition of LR , L and R for PET quantification: $[LR] = B$ (bound radioligand concentration), $[L] = F$ (free radioligand concentration) und $[R] = B_{avail}$ (free available receptors). B_{avail} arises from the deviation of B_{max} and B :

$$B_{avail} = B_{max} - B \quad [6]$$

Insertion in equation 4:

$$K_d = \frac{k_{off}}{k_{on}} = \frac{F \times B_{avail}}{B} = \frac{F(B_{max} - B)}{B} \quad [7]$$

$$K_d B = F(B_{max} - B) = FB_{max} - FB \quad [8]$$

$$K_d B + FB = FB_{max} \quad [9]$$

$$B(K_d + F) = FB_{max} \quad [10]$$

$$B = \frac{FB_{\max}}{(K_d + F)} \quad (\text{Michaelis-Menten-Equation}) \quad [11]$$

Under the assumption that the tracer principle is valid, i.e. $F \ll K_d$:

$$B = \frac{B_{\max}F}{K_d} \Leftrightarrow \frac{B}{F} = \frac{B_{\max}}{K_d} \quad [12]$$

The definition of the binding potential depends on the definition of the reference region. The notation BP_{ND} is used, if the binding potential refers to a region without specific binding sites (**n**on **d**isplaceable) and BP_P and BP_F are used if it refers to the total or free plasma concentration (Innis et al., 2007). Under *in vivo* conditions, only the free fraction of the radioligand in tissue f_{ND} (or plasma, f_P) is available for specific binding. Therefore the BP_{ND} under *in vivo* conditions is defined by the following equation:

$$k_3 = f_{ND}k_{on}B_{avail} \quad [13]$$

$$k_4 = k_{off} \quad [14]$$

Insertion in equation 12 results in:

$$BP_{ND} = \frac{k_3}{k_4} = \frac{f_{ND}k_{on}B_{avail}}{k_{off}} = \frac{f_{ND}B_{avail}}{K_d} \quad [15]$$

Distribution Volume. The distribution volume (V_T) is defined as the ratio of C_T and C_P and describes not a true volume, but a virtual “volume”, which the radioligand would capture, if it had the same concentration in tissue as in plasma. V_{ND} describes the V_T of the non displaceable ligand in tissue relative to the plasma concentration ($V_{ND} = V_F + V_{NS}$) (Innis et al., 2007).

$$V_T = \frac{C_T}{C_P} \quad [16]$$

$$V_{ND} = \frac{C_{ND}}{C_P} \quad [17]$$

Relation between BP and V_T (Innis et al., 2007). Since the specific binding is defined as the difference of V_T and V_{ND} , the binding potential (BP) can be defined as:

$$BP_{ND} = \frac{V_T - V_{ND}}{V_{ND}} = \frac{V_T}{V_{ND}} - 1 = DVR - 1 \quad [18]$$

The ratio of V_T/V_{ND} is called the distribution volume ratio (DVR). Therefore the BP can be estimated from the ratio of specifically bound and free radioligand under equilibrium conditions. However, in most PET experiments, the radioligand is injected

as single bolus and thus equilibrium conditions are not achieved during the PET experiment. In those experiments, the binding parameters are estimated from the rate constants which were estimated from the dynamic PET data. Over the past decade, a number of PET tracer models have been developed for the estimation of quantitative parameters. These methods can be divided into two categories: *model dependent* methods und *data dependent (model independent)* methods (Gunn et al., 2002).

Model dependent methods

Model dependent methods refer to a specific compartmental structure, which describes the behaviour of the radioligand in tissue and estimates either micro- (K_1 , k_2) or macro-constants (BP_{ND} , V_T). The behaviour of the radioligand in the different compartments is described by linear differential equations. For a *1-compartment-model* from figure 2, the concentration in C_{F+NS+S} ($= C_1$) is defined as:

$$\frac{dC_1(t)}{dt} = K_1 C_p(t) - k_2 C_1(t) \quad [19]$$

$C_p(t)$ is the change of radioligand concentration in blood plasma, K_1 the rate constant which describes the transfer of the radioligand from blood into tissue and k_2 the rate constant which describes the transfer from tissue back into the blood. K_1 is written in capital letters, since it has a different unit in comparison to all other rate constants. While radioactivity concentrations in blood have the unit kBq/mL, the tissue concentration is defined in kBq/g. Therefore, K_1 has the unit mL/min/g, while all the other rate constants have the unit min^{-1} .

Convolution

Consider an idealized system in which an infinitesimal impulse (delta function), proportional to K_1 has reached the first compartment, under the assumption that $C_p = 0$ (i.e. the compartment has an initial activity without further input during the experiment = ideal bolus) (figure 3A). In this case, equation 19 has the following solution in the case of a 1-compartment-model:

$$C_1(t) = C_p K_1 e^{-k_2 t} \quad [20]$$

Consider now the tissue activity responses to many single boli inputs at different time points T_i and different amplitudes $C_p(T_i)$ (figure 3B,C), then the tissue response can mathematically be described as a sum of individual responses. The responses are shifted in time and scaled in magnitude:

$$C_1(t) = \sum_i C_p(T_i) K_1 e^{-k_2(t-T_i)} \quad [21]$$

Suppose now a continuous input function C_p as sum of a series of bolus administrations at times T_i (figure 3D), then the sum from equation 21 becomes an integral:

$$C_1(t) = \int_0^t C_p(s) K_1 e^{-k_2(t-s)} ds \quad [22]$$

This integral is called the convolution integral from plasma input and system response:

$$C_1(t) = C_p(t) \otimes K_1 e^{-k_2 t} \quad [23]$$

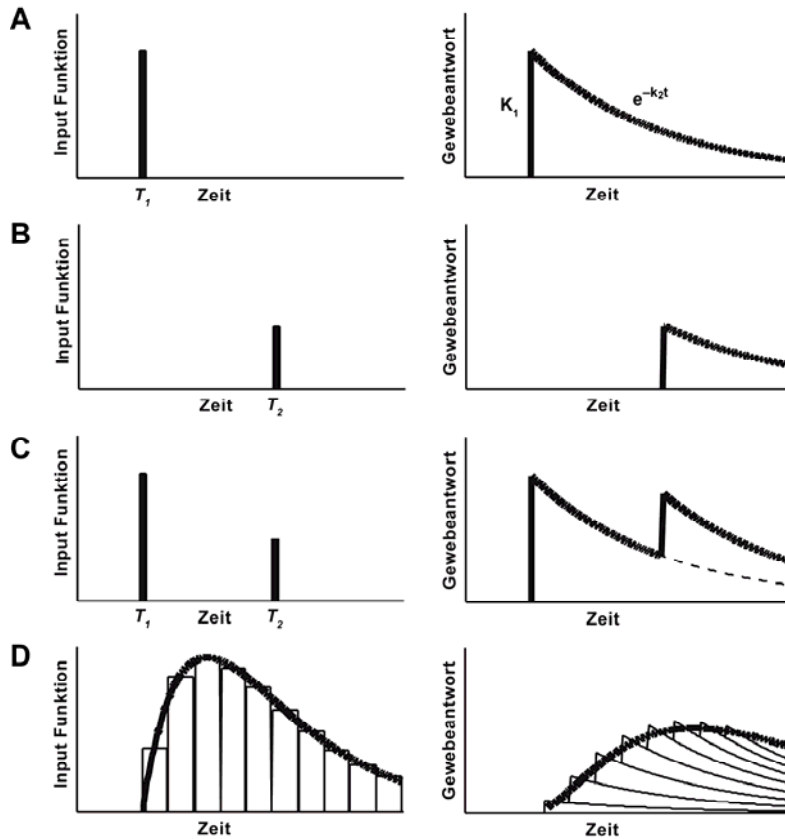


Figure 3: Graphical illustration of the convolution of plasma input (left) and impulse-response-function to the tissue response function (right). **A** Tissue response to an infinitesimal impulse (delta function), proportional to K_1 at the time point T_1 , **B** tissue response to an infinitesimal impulse at time point T_2 with small amplitude, **C** tissue response to two single boli at time point T_1 and T_2 from summation of single responses, **D** tissue response (time activity curve) from a continuous input function arising from the summation of single responses. Modified from Carson (2005).

For a 2-compartment model ($C_{F+NS} = C_1$, $C_S = C_2$) (figure 2), the differential equations are written as follows:

$$\frac{dC_1(t)}{dt} = K_1 C_p(t) - (k_2 + k_3) C_1(t) + k_4 C_2(t) \quad [24]$$

$$\frac{dC_2(t)}{dt} = k_3 C_1(t) - k_4 C_2(t) \quad [25]$$

The number of compartments corresponds to the number of exponentials (Gunn et al., 2001):

$$C_T(t) = (\phi_1 e^{-\theta_1 t} + \phi_2 e^{-\theta_2 t}) \otimes C_P(t) \quad [26]$$

$$\phi_1 = \frac{K_1 \times (\theta_1 - k_3 - k_4)}{\Delta} \quad \theta_1 = \frac{k_2 + k_3 + k_4 + \Delta}{2}$$

$$\phi_2 = \frac{K_1 \times (\theta_2 - k_3 - k_4)}{-\Delta} \quad \theta_2 = \frac{k_2 + k_3 + k_4 - \Delta}{2}$$

$$\Delta = \sqrt{(k_2 + k_3 + k_4)^2 - 4 \times k_2 \times k_4}$$

with the macro-constants θ (decay constant) and Φ (amplitude), which result from the micro-constants K_1, k_2, k_3, k_4 . The estimated parameters are received from linear and non-linear fitting methods.

Examples for model dependent methods are the Kety-Schmidt-Model for the quantification of blood flow in the brain (Kety and Schmidt, 1948), the 2-compartment-model for the quantification of glucose consumption in the brain (Sokoloff, 1981) and the reference tissue model, for example the *Four Parameter Reference Tissue Model* (4PRTM) (Hume et al., 1992) and the *Simplified Reference Tissue Model* (SRTM) (Lammertsma and Hume, 1996).

Data dependent methods

Data dependent methods are derived from linearization of differential equations (Gjedde, 1982; Logan et al., 1996; Logan et al., 1990; Patlak et al., 1983). They do not allow a full characterization of the tissue regarding all rate constants, but are limited to the estimation of macro parameters, like the V_T or the BP (equation 14-17).

Quantification of receptor binding

The Simplified Reference Tissue Model is described by the following equation (Lammertsma and Hume, 1996):

$$C_T(t) = R_1 C_R(t) + k_2' \left(1 - \frac{R_1}{1 + BP_{ND}} \right) e^{\left(\frac{-k_2'}{1 + BP_{ND}} \right) t} \otimes C_R(t) \quad [27]$$

The parameters R_1, k_2 und BP_{ND} can be estimated by non-linear curve fitting.

Quantification of regional cerebral blood flow

Quantifying regional cerebral blood flow in small laboratory animals like mice is of utmost interest for preclinical applications ranging from oncology and immunology to neurology. However, this technique features several severe problems that will be discussed in the following paragraphs.

As we are interested in assessing regional cerebral blood flow, flow tracers should ideally mimic freely diffusible biomolecules with water being the most obvious among

possible candidates as the intrinsic *in vivo* concentration in blood is very high (~50M) and thus, the system is not violated by further injections of small amounts of water. In PET applications, the intrinsic oxygen atom (^{16}O) is replaced by a positron emitting isotope (^{15}O) rendering PET measurements possible. However, ^{15}O has a very short half-life of only 122 sec, exacerbating tracer handling. Furthermore, blood flow is affected by various parameters including anesthesia, blood gases or in general the physiologic state of the measured animal. This means that exact biomonitoring of vital parameters is extremely important.

For [^{15}O]H₂O-PET measurements, knowing the absolute tracer concentration in the respective tissue of interest and in the vascular space (to be more precise: in the arterial space) is mandatory for absolute quantification of blood flow. In general, blood flow values are given in ml per minute per 100 g tissue being perfused (ml/min/100g) and range from 50 ml/min/100g in humans to 200 ml/min/100g in mice. In humans, measuring the arterial tracer concentration is feasible as the surgery can be easily done (a catheter has to be inserted in a distal artery like the radialis in the forearm). The blood flows in this case from the arterial space in the patient through the catheter towards a second very small PET detector (blood counter). After blood counting, the blood is not flowing back into the patient's vascular system. However, in mice this method is not feasible as the total blood volume of a 20 g laboratory mouse is approximately only 2 ml and thus discarding the blood drawn from the animal is not an option. As all anatomical structures in mice like arteries are very small, surgeries are rendered very difficult. Current solutions to this problem are PET-image derived arterial input functions that are used for the following modelling. For perfusion measurements with [^{15}O]H₂O, a single compartmental model is used for the calculation of blood flow. The following differential equation describes the model system regarding [^{15}O]H₂O-PET studies:

$$\frac{dC_t(t)}{dt} = K_1 C_p(t) - k_2 C_t(t)$$

$\frac{dC_t(t)}{dt}$: time derivative of tissue concentration

$C_p(t)$: tracer concentration in plasma

$C_t(t)$: tracer concentration in tissue

K_1 : blood flow

k_2 : blood flow corrected with partition coefficient

This differential equation is solved and the solution further used for the fitting of kinetic constants describing the model system (K_1 and k_2). Moreover, the resulting operational equation used for the fitting routine has to be corrected for tracer arrival delay and dispersion. Tracer arrival delay is caused by the time the tracer needs to travel from the heart to the tissue of interest (in the case of an image derived arterial input function). Dispersion is the broadening of the time activity curve as the tracer interacts with the vascular wall. So far, this approach has been validated in humans and rats while it is still not fully evaluated in mice.

Quantification of glucose consumption

FDG (Fluorodeoxyglucose), a glucose analog, is the most widely used radiotracer in PET. It can be taken by the cells through glucose transporters and phosphorylated into FDG-6-P by hexokinase. However, FDG-6-p can't be further metabolized like glucose-6-P and hence accumulates in the cells. In order to describe the FDG kinetic process, a 2-tissue compartment model has been developed:



$$\begin{aligned}\frac{dC_F}{dt} &= K_1 C_P - k_2 C_F - k_3 C_F + k_4 C_M \\ \frac{dC_M}{dt} &= k_3 C_F - k_4 C_M\end{aligned}$$

where C_p is the concentration of free FDG in plasma. C_F and C_M are not structural compartments but different states of the tracer in the tissue. C_F is the concentration of free FDG in tissue, and C_M is the concentration of metabolized FDG (FDG-6-P) in tissue. K_1 (ml/min/ml) and k_2 (min^{-1}) are the rate constants of FDG transport into cells or back to plasma respectively. k_3 (min^{-1}) and k_4 (min^{-1}) are the rate constants of phosphorylation of FDG and dephosphorylation of FDG-6-P respectively. The total tracer concentration in tissue, C_T , can be solved from the above equations:

$$\begin{aligned}C_T(t) &= C_F(t) + C_M(t) \\ &= \frac{K_1}{\alpha_2 - \alpha_1} \left[(k_3 + k_4 - \alpha_1) e^{-\alpha_1 t} + (\alpha_2 - k_3 - k_4) e^{-\alpha_2 t} \right] \otimes C_p(t) \\ \alpha_1, \alpha_2 &= \frac{k_2 + k_3 + k_4 \mp \sqrt{(k_2 + k_3 + k_4)^2 - 4k_2 k_4}}{2}\end{aligned}$$

where $\otimes$ denotes convolution operation.

C_T and C_p are derived from several time points and compose time-activity curves (TAC). The interest of the kinetic model is to determine the k_n values from TAC fitting by applying the mathematical equation and further estimate the glucose metabolic rate (MRGlc, $\mu\text{mol}/100\text{g}/\text{min}$):

$$\text{MRGlc} = \frac{Ki \cdot C_{glu}}{LC}$$

where $Ki = K_1 k_3 / (k_2 + k_3)$

C_{glu} is the concentration of glucose in plasma, and LC is the lumped constant which corrects different transport and phosphorylation rates between glucose and FDG.