Nonlinear Mixed Effects Methods for Improved Estimation of Receptor Occupancy in PET Studies

Matts Kågedal

Receptor occupancy assessed by Positron Emission Tomography (PET) can provide important translational information to help bridge information from one drug to another or from animal to man. The aim of this thesis was to develop nonlinear mixed effects methods for estimation of the relationship between drug exposure and receptor occupancy for the two mGluR5 antagonists AZD9272 and AZD2066 and for the 5HT₁B receptor antagonist AZD3783. Also the optimal design for improved estimation of the relationship between drug exposure and receptor occupancy as well as for improved dose finding in neuropathic pain treatment, was investigated.

Different modeling approaches were applied. For AZD9272, the radioligand kinetics and receptor occupancy was simultaneously estimated using arterial concentrations as input function and including two brain regions of interest. For AZD2066, a model was developed where brain/plasma partition coefficients from ten different brain regions were included simultaneously as observations. For AZD3783, the simplified reference tissue model was extended to allow different non-specific binding in the reference region and brain regions of interest and the possibility of using white matter as reference was also evaluated. The optimal dose-selection for improved precision of receptor occupancy as well as for improved precision of the minimum effective dose of a neuropathic pain treatment was assessed, using the D-optimal as well as the Ds-optimal criteria.

Simultaneous modelling of radioligand and occupancy provided a means to avoid simplifications or approximations and provided the possibility to tests or to relax assumptions. Inclusion of several brain regions of different receptor density simultaneously in the analysis, markedly improved the precision of the affinity parameter. Higher precision was achieved in relevant parameters with designs based on the Ds compared to the D-optimal criterion. The optimal design for improved precision of the relationship between dose and receptor occupancy depended on the number of brain regions and the receptor density of these regions.

In conclusion, this thesis presents novel non-linear mixed effects models estimating the relationship between drug exposure and receptor occupancy, providing useful translational information, allowing for a better informed drug-development.

**Keywords:** PET, positron emission tomography, receptor occupancy, nonlinear mixed effects, NONMEM, optimal design, dose finding

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Till min familj
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


III Kågedal M, Varnäs K, Hooker AC, Karlsson MO. Extension of the simplified reference tissue model, to allow different non-specific concentrations in the brain region of interest and the reference region. Application to PET with the radioligand $^{11}$C]AZ10419369 displaced by AZD3783 at the serotonin 5-HT$_{1B}$ receptor. (submitted)

IV Kågedal M, Karlsson MO, Hooker AC. Improved precision of exposure-response relationships by optimal dose-selection. Examples from studies of receptor occupancy using PET and dose finding for neuropathic pain treatment. (in manuscript)

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Abbreviations and Nomenclature

BBB Blood Brain Barrier
ΔOFV Difference in OFV; likelihood ratio
BMAX Maximum binding capacity, usually corresponding to the receptor density
BPBL BPND at the baseline PET measurement (tracer amount of radioligand only)
BPND Binding potential corresponding to the ratio of the specifically bound to the non-displaceable concentration in the brain (CB/CND) at equilibrium
CAU Caudate
CB Concentration bound to receptors (specific concentration)
CBLOOD Blood concentration
CER Cerebellum
CND Non-displaceable concentration (free+ non-specific binding)
Cp Concentration in plasma
CPET Plasma concentration of unlabeled displacer during the PET-measurement
CSS Plasma concentration at steady state
CV Coefficient of variation
D-optimal Optimal design criterion to improve overall precision of model parameters
Ds-optimal Optimal design criterion to improve precision of specified model parameters of interest
E Efficacy
E*,C* The parameters C* and E* is a concentration effect pair on the concentration response curve, one being chosen appropriately and the other being an estimated model parameter
EC50 The Css that produces half of EMAX
EMAX The maximum effect attributable to the drug
EPLACEBO Placebo response
FIM Fisher information matrix
K1 Rate-constant determining the transfer rate from plasma to the brain
k2 Rate-constant determining the transfer rate from brain to
plasma

$k_3$ Rate constant corresponding to $k_{on} \times R_{FREE}$

$K_{dND}$ Affinity constant corresponding to the non-displaceable CNS concentration at 50% occupancy

$K_{dPL}$ Affinity constant corresponding to drug-concentration inducing 50% occupancy.

$K_{iPL}$ Affinity constant corresponding to the plasma concentration at 50% occupancy

$k_{off}$ Rate constant for dissociation from the receptor

$k_{on}$ Rate constant for binding to the receptor

$K_{P_{ND}}$ Partition coefficient corresponding to $C_{ND}/C_p$ at equilibrium ($V_{ND}$ in PET-standard terminology)

$K_{P_{REF}}$ $K_{P_{ND}}$ of the reference region

$K_{P_S}$ Specific brain to plasma partition coefficients

$K_{P_{S0}}$ Specific brain to plasma partition coefficient at tracer condition

$K_T$ Partition coefficient corresponding to the ratio of total radioactivity concentration in the brain and concentration of unchanged radioligand in plasma at equilibrium ($V_T$ in PET-standard terminology)

$K_{P_{T,BL}}$ $K_T$ at the baseline PET measurement (no pre treatment)

$K_{P_{T,PT}}$ $K_T$ obtained after pretreatment with drug that competes with the radioligand for the receptor binding

$mGluR5$ Metabotropic glutamate receptor subtype 5

MRI Magnetic Resonance Imaging

$ND_{REL}$ The non-displaceable brain-plasma partition coefficient of the ROI ($K_{P_{ND}}$) relative to that in the reference region ($K_{P_{REF}}$)

NLME Nonlinear mixed effects models

NRS Numerical rating scale (0-10) for assessment of pain level

PET Positron emission tomography

$R_1$ Parameter of the SRTM accounting for the difference in equilibration rate between the reference region and a ROI

$RBV$ Relative blood volume derived as the brain blood volume divided by the total brain volume

REF Brain reference region

$R_{FREE}$ Free receptor concentration

RMSE Root mean square error

ROI Brain region of interest

RSE Relative standard error (standard error / parameter estimate)

$SD$ Standard deviation

SRTM Simplified reference tissue model
VST Ventral striatum
α (alpha) \(1/EC_{50}\)
ε (epsilon) Difference between individual prediction and observation (residual error)
η (eta) Difference between population and individual parameter estimate
γ (gamma) Slope factor, determining the steepness of the Emax curve
Θ (theta, large caps) Model parameters
θ (theta) Fixed-effect parameter (typical value)
σ (sigma) Standard deviation of ε
ω (omega) Standard deviation of η
Introduction

In drug development a good understanding of the relationship between dose and efficacy as well as safety will result in improved dose selection for clinical trials, and ultimately to better dosing recommendations for patients. The understanding of the relationship between dose and clinical effects is limited early in the development of a new drug. Often however information exists on preclinical pharmacology and on the pharmacological effects of other drugs in man with a similar mechanism of action. To make use of this information, a means to translate the results from the preceding drug to the drug under development or from the preclinical species to man is needed. Receptor occupancy by means of Positron Emission Tomography (PET) provides such translational information and can hence improve predictions of dose response and make drug-development more efficient. PET-studies are technically challenging to perform and each PET-measurement is very costly. Ensuring that the study designs are informative based on relatively few measurements, and that the method of data-analysis is as effective as possible in extracting that information is therefore important.

The development of novel approaches based on non-linear mixed effects methods (NLME), intended to improve the design and analysis of receptor occupancy studies using PET is the subject of this thesis.

Receptor occupancy as a translational tool

Most drugs mediate their effect by binding to a specific binding site (target) on receptors, ion channels, transporters or enzymes. Assessment of the extent of target binding as biomarker, being on the mechanistic path between drug exposure and response, can support predictions of therapeutic response. Translation can occur from \textit{in vitro} to \textit{in vivo}, from animal to man as well as between drugs with the same target\(^1\). When receptor occupancy data is not available for a drug targeting the brain, predictions are often based on translation via drug-concentration in plasma. For these predictions to be valid any differences between drugs or species in plasma protein binding, transport across the blood brain barrier (BBB) and affinity to the receptor needs to be taken into account\(^2\). These factors can vary considerably between drugs and species\(^3\) and are often challenging to estimate accurately. Assessment of
receptor occupancy using PET avoids these problems, giving a measure of the extent of target binding in vivo at the site of action.

Occupancy corresponds to the fraction of binding sites occupied by the drug molecule. The occupancy required to induce clinical effects depends on the target as well as the molecule. The most documented case is that for antipsychotics where a relationship between receptor occupancy and efficacy as well as extrapyramidal side-effects has been established both for typical and atypical antipsychotics. In 1992, Farde et al. showed that for classical antipsychotics, D2 receptor occupancy of approximately 70-80% was efficacious with low risk for extrapyramidal side effects. Later it was shown that therapeutic doses for atypical antipsychotics was lower at around 65%-7. A review by Grimwood and Hartig show that for antagonists binding to GPCRs, ion channels or transporters a relatively high occupancy in the range 60-90% appears to be required for a therapeutic effect. For agonists the therapeutic occupancy is more variable and depends on the intrinsic activity of the agonist, the receptor or ion channel reserve as well as the response that is measured.

Positron Emission Tomography

Positron emission tomography (PET) is a non-invasive technique that allows imaging of a molecule labelled with a radionuclide in the living brain. Positron-emitting radionuclides such as [11C]-carbon, [13N]-nitrogen and [15O] are available. Substitution of a stable isotope by the positron-emitting equivalent makes it possible to track endogenous compounds as well as drugs in vivo. Radioligands, binding to specific targets have been developed as biomarkers to support the diagnosis of diseases such cancer, Alzheimer as well as Parkinson’s disease. The focus of the present thesis is however on the investigation of drug binding to receptors in the brain.

After intravenous injection, the radiolabelled molecule (radioligand) distributes via the bloodstream to all parts of the body and passes the blood-brain-barrier into the brain. Positrons are emitted from the radioisotope which has been incorporated in the ligand. Within a few mm, the positron interacts with an electron and annihilation occurs in which two photons are emitted in opposite directions. The photons are subsequently detected by a coincidence detector system of the PET-camera. By the use of a series of corrections and complex mathematical calculations, the radioactivity counts can be reconstructed into a time series of three-dimensional images of radioligand concentrations. Based on this information the time-course of radioligand concentration can be derived for different regions of the brain. The PET-data can be combined with magnetic resonance imaging to support the definition of anatomical brain regions of interest.
Radioligand models and receptor occupancy

Occupancy of a drug can be assessed by analysis of radioligand concentration data in different regions of the brain, derived by PET. Different modelling and non-modelling approaches have been applied to derive measures of the specific binding of the radioligand and occupancy. Compartmental models using radioligand concentration in arterial plasma as input function is commonly used\(^\text{12}\) and is often considered as the gold standard\(^\text{13}\). In some cases a region in the brain can be identified that lacks the target receptor. The nonspecific concentrations of the region of interest has then been approximated with the concentrations of the reference region\(^\text{14, 15}\). However approaches to account for the lack of binding equilibrium have later been proposed\(^\text{15-17}\). Graphical analysis where the data are first transformed to allow the parameter of interest to be estimated from a subsequent linear regression is also sometimes used\(^\text{18-21}\). These methods can be useful in particular for voxel level analysis where more computationally demanding methods may not be feasible. The graphical methods can however be biased by statistical noise\(^\text{22}\). Several common quantification models are described in a recent review by Varnäs et al\(^\text{23}\). When no reference region lacking receptors exists, the estimation of occupancy is more difficult. Inclusion of more than one brain region in the analysis can then be informative, leveraging the differences in specific uptake between brain regions\(^\text{24}\). The standard process for the analysis of radioligand concentration data from PET to derive the relationship between drug-exposure and occupancy involves a sequence of separate steps. In the first step, the radioligand kinetics is determined based on each individual PET measurement. The individual rate constants of the kinetic model are not usually identifiable, but macro parameters providing an index of the level of specific receptor binding, the binding potential (BP\(_{\text{ND}}\)) or the total brain/plasma partition coefficient (KP\(_{\text{T}}\)) can usually be derived\(^\text{25, 26}\). Based on these macro parameters the reduction in specific binding (occupancy) is derived by relating each pre-treatment PET to the baseline PET. In a last step, the relationship between occupancy and drug-exposure is derived. For targets where a reference region exists, the macro parameter used is usually BP\(_{\text{ND}}\)\(^\text{14, 27-30}\) and when no reference region exists the KP\(_{\text{T}}\) can be used\(^\text{31}\).

Nonlinear Mixed Effects Modeling

Nonlinear mixed effects (NLME) is a useful tool extensively used in the analysis of pharmacokinetic and pharmacokinetic-pharmacodynamic data in drug-development\(^\text{32-36}\). In NLME analyses, data from a group of subjects is analyzed and both the typical parameters (fixed effects) and the variability (random effects) are simultaneously quantified. The function defined by the fixed effects in the model describes a typical subject in the population. Diff-
ferent levels of random effects are included in the model to account for variability between individuals and the unexplained residual error. It is also possible, and sometimes important to account for variability between occasions in an individual subject\textsuperscript{37, 38}. In NLME analyses, all subjects are contributing with information to the model and the information is shared between the subjects. This can be particularly useful if information on parameters is sparse in individual subjects\textsuperscript{39} and if the design is unbalanced\textsuperscript{40} which is often the case in PET studies. Also, when analyzing PET-data, different parts of the brain can contribute with different information making it particularly useful to include more than one region simultaneously in the analysis.

The parameter estimates of a model are found by iteratively searching for the parameter estimates that maximize the likelihood of the observations given the model. This is done by minimizing the objective function (OFV), which is approximately proportional to \(-2\cdot\text{log-likelihood of the data}\). A difference in the OFV of 3.84 corresponds to a p-value of 0.05 for a one-parameter difference between nested models (if the OFV is exactly \(-2\cdot\text{log-likelihood of the data}\)). In later years, non-linear mixed effects models for the analysis of PET data has increasingly been used\textsuperscript{41-48}. These analyses show that NLME modelling can be successfully applied to PET data and improves the understanding of variability. It is also suggested that the statistical power for estimation of binding parameters is improved and that it is possible also to include individuals with incomplete data in the analysis.

Optimal design for improved dose finding

Dose selection in early clinical trials and ultimately for phase III represents a major challenge in drug development. In some cases just detecting a difference from placebo is difficult due to limited treatment effects and large variability in response, making a more detailed description of the shape of the curve very difficult. In addition, the underlying relationship between exposure and response is not usually well known making it difficult to define possible underlying relationships for which the study design needs to be optimized. Nevertheless, the importance of a good understanding of dose response is stressed in regulatory guidelines\textsuperscript{49, 50}. In recent years, methods for improved design of dose finding studies and for receptor occupancy studies using PET have been proposed\textsuperscript{51-55}. In the present thesis the optimal dose-selection for two different hypothetical studies are investigated. The first one is a receptor occupancy study assessed by positron emission tomography (PET) and the second one is a phase IIb dose-finding study of a drug aimed for neuropathic pain treatment.

When the biologic system influenced by the drug is well understood a mechanistic model can be applied where the parameters have a biological meaning and therefore can be of interest. In the case of the receptor occu-
pancy study, the applied Emax model has a mechanistic basis in the law of mass action. For understanding the exposure-response relationship, then, the interest is mainly focused on the affinity parameter of the model (KiPL). Occupancy is never directly measured in the study, but can be inferred at any plasma concentration based on this one parameter.

In contrast to the mechanistic model described above, for many cases, the system influenced by the drug is poorly understood. This is the situation for the Phase IIb dose-finding study considered in this thesis. Also here an Emax model has adequate properties and has been applied before to describe exposure response for neuropathic pain treatments. In this situation however, the model should be regarded as empirical. That is, the model parameters do not have a meaning in terms of biological processes and it is the precision of the response curve rather than the model parameters that is of relevance. Not all parts of the curve are of equal importance however. The dose(s) for phase III confirmatory studies will most likely be selected somewhere in the range from the minimum clinically relevant effective dose (MED) up to the maximum tolerated dose, making it particularly relevant to improve the precision of this part of the curve. An alternative parameterization of the Emax model has been proposed where the concentration or dose corresponding to the minimum clinically relevant effect can be estimated as a parameter of the model. Optimizing the study design (e.g. dose levels) with regards to the precision of that parameter will thus have a direct relevance for defining the minimum effective dose.

In optimal design theory different criteria based on the fisher information matrix (FIM) can be used to optimize experiments. One of the more common is the D-optimal design criterion with which the overall precision of parameters is maximized. When the interest is limited to a subset of the parameters, the Ds-optimal criterion, offers an alternative, where the design can be optimized for improved precision of these interesting parameters, while still acknowledging that all parameters will be estimated.
The aim of the research presented in this thesis was to develop methods that improve precision, allow more powerful tests of assumptions, improve understanding of variability and reduce the need for simplifications/approximations in receptor occupancy studies by PET.

Specific aims were:

- To develop, in the absence of a reference region, a model that simultaneously estimates radioligand kinetics and receptor occupancy, including two brain regions of interest.

- To develop a model that allows estimation of occupancy by inclusion of many brain regions of interest using the derived brain to plasma partition coefficients as the dependent variable (observation).

- To develop an extension to the simplified reference tissue model, allowing non-displaceable binding to differ between the reference region and brain regions of interest, providing the possibility to use white matter as a reference region.

- To investigate, by means of optimal design methodology, the optimal allocation of doses for improved precision in the exposure-occupancy relationship for a receptor occupancy study and a dose-finding study of a drug intended for neuropathic pain treatment.
Material and Methods

While the radioligands and the drugs differed between the PET studies, the study designs and the method of measurement were similar. The approach for the analysis did however differ. In the sections below, the theoretical principles are first introduced. Subsequently the study designs and method of measurement are described. Thereafter a description of the study drugs and radioligands involved is given and lastly the implementations of the models are described.

Theory on drug and radioligand kinetics and receptor binding

The basic model for the radioligand kinetics and binding derived from the two tissue-compartment model proposed by Mintun et al.\textsuperscript{12}, is illustrated in Figure 1. After intravenous injection, the radioligand distributes among physical and chemical compartments. The major compartments are plasma (C\textsubscript{p}), non-displaceable (C\textsubscript{ND}), and specifically bound to radioligand receptors (C\textsubscript{B}). The observed arterial plasma concentrations are usually included as input function and not modelled.

\begin{figure}[h]
\centering
\includegraphics[width=0.6\textwidth]{model.png}
\caption{Standard two tissue compartment model describing drug uptake and binding of the radioligand}
\end{figure}
The rate of change in $C_{ND}$ and $C_B$ can be described by the following differential equations:

\[
\frac{dC_{ND}}{dt} = K_1 \cdot C_p - k_2 \cdot C_{ND} - k_{on} \cdot [R_{Free}] \cdot C_{ND} + k_{off} \cdot C_B
\]

\[
\frac{dC_B}{dt} = k_{on} \cdot [R_{Free}] \cdot C_{ND} - k_{off} \cdot C_B
\]

where $K_1$ and $k_2$ are rate-constants determining the transfer rate between plasma and brain. The free receptor concentration is $[R_{Free}]$ and $k_{on}$ and $k_{off}$ are the rate-constants for binding to and dissociation from the receptors. The radioactive concentration in a brain region registered by the PET camera over time corresponds to the sum of the non-displaceable and specifically bound concentrations. In addition concentration in the blood ($C_{BLOOD}$) contributes to the detected signal. The total concentration in the region of interest ($C_{ROI}$) corresponds to

\[
C_{ROI} = (C_{ND} + C_B) \cdot (1 - RBV) + (C_{BLOOD} \cdot RBV)
\]

where RBV is the relative blood volume in CNS, assumed to be 5% in the present work.

**Macro parameters**

The individual parameters of the model are sometimes estimated with poor precision and in some cases they are not identifiable at all. When this is the case, robust estimates of macro parameters can often still be obtained. The derivation of common macro parameters are provided below.

The partition coefficient corresponding to the ratio of total radioactivity concentration in the brain and concentration of unchanged radioligand in plasma at equilibrium ($K_{PT}$) can be derived from the parameters of the two tissue compartment model (Eq. 1) as

\[
K_{PT} = \frac{K_1}{k_2} \cdot (1 + \frac{k_{on} \cdot R_{FREE}}{k_{off}})
\]

The $K_{PT}$ can also be expressed as

\[
K_{PT} = K_{PS} + K_{PND}
\]

where $K_{PS}$ and $K_{PND}$ are the specific and non-displaceable brain to plasma partition coefficients respectively. The $K_{PT}$ can also be derived without making assumptions on the compartmental structure using the graphical method.
proposed by Logan et al\textsuperscript{18}. The partition coefficient corresponding to $C_{ND}/C_p$ at equilibrium ($K_{PND}$) can be calculated from

\begin{equation}
K_{PND} = \frac{K_1}{k_2}
\end{equation}

In PET-literature the partition coefficients are often referred to as volumes of distribution\textsuperscript{26}. In the present thesis, the term partition coefficient is used similarly as in physiologically based pharmacokinetic modelling. The binding potential ($BP_{ND}$) is frequently used as an index of receptor binding. $BP_{ND}$ corresponds to the ratio of the specifically bound to the non-displaceable concentration in the brain ($C_B/C_{ND}$) at equilibrium and can be derived from

\begin{equation}
BP_{ND} = \frac{k_{on} \cdot R_{FREE}}{k_{off}}
\end{equation}

or from

\begin{equation}
BP_{ND} = \frac{K_P}{K_{PND}}
\end{equation}

When each PET measurement is modelled separately after tracer doses of the radioligand, the $K_{on}$ and $R_{FREE}$ are not identifiable, instead the parameter $k_3$ corresponding to the product of $K_{on}$ and $R_{FREE}$ is estimated.

**Optimal design methods**

The FIM contains information on parameter precision (lower bound on covariance of parameter estimates) and can be derived based on the model, the parameters ($\Theta$) and the design variables ($q$). For nonlinear mixed effects models, the FIM must be approximated because the marginal likelihood is generally not solvable. An initial approximation for random effects models was proposed by Mantré et al\textsuperscript{60}. In the present work, the first order approximation of the FIM for population models described by Foracchia, Hooker et al. (2004) was used\textsuperscript{61}. Study designs were optimized based on the D and Ds optimal design criteria. With the D-optimal criterion, the overall precision of model parameters is improved by maximizing the determinant of the FIM (Eq. 8). The Ds-criterion allows the definition of interesting parameters for which the precision is to be maximized. In this case the precision of the interesting parameters is improved by maximizing a ratio between the determinant of the FIM for all parameters in the model and the determinant of the FIM for the parameters defined as not interesting (NS) (Eq. 9).
The $D_s$-optimal design offers a better alternative to fixing parameters that are of no or low interest. When a parameter is fixed, it is removed from the FIM. The $D_s$ criterion on the other hand, acknowledges that the parameter will be estimated and accounts for possible correlations with other parameters in the model\textsuperscript{62}.

**Studies and study designs**

Papers I-III are based on data obtained from studies in healthy male subjects. The studies were performed in accordance with the Declaration of Helsinki and Good Clinical Practice. Written informed consent was obtained from all subjects. Paper IV dealt with optimal designs and did not include any observed data.

The same basic study design was used in the three PET studies of paper I-III and also as applicable for the work on optimal design of a PET-study in paper IV. All studies included six healthy males, which were planned to participate in four PET measurements each. Each volunteer was also examined by Magnetic Resonance Imaging (MRI) used for delineations of anatomical regions of interests (ROIs). The first PET measurement was a baseline assessment including intravenous administration of tracer amounts of a radioligand alone. The three subsequent PET examinations were performed after pre-treatment of different single oral doses of the drug under investigation (the displacer). The PET measurements were started at around three hours post administration of the displacer which was after the time of maximum plasma concentration of the displacer.

Paper IV also included work on the optimal dose-selection for a dose-finding study in neuropathic pain. The assumed design of that study was a four-armed balanced study design (n=54/arm) including a placebo and the maximum tolerated dose. The two mid-doses were to be defined in the computations of the optimal design.

**PET Measurements**

Following intravenous injection of the radioligand, brain radioactivity was measured with the PET system in a consecutive series of time frames during a total acquisition time of 60-90 minutes (depending on the study). The delineations of anatomical brain regions were made manually on the reoriented
MR images using the Human Brain Atlas software\textsuperscript{48}. The radioactivity concentration in each brain ROI was calculated for each sequential frame and corrected for radioactive decay.

The AZD9272 and AZD2066 studies (paper I, II) included blood sampling from arteria radialis which were collected and analyzed, as described before\textsuperscript{15, 63} in order to derive unchanged (metabolite corrected) radioligand plasma concentrations over time. Venous blood samples were drawn for determination of the plasma concentration of the unlabeled drug for which occupancy was to be determined (i.e. AZD9272, AZD2066 or AZD3783) before, during and after completion after PET data acquisition.

Study drugs and radioligands

The metabotropic glutamate receptor subtype 5 (mGluR5) is widely distributed in the central nervous system and has received attention as a potential therapeutic target in various diseases such as anxiety, depression, and pain disorders\textsuperscript{64-68}. Two high affinity mGluR5 antagonists, AZD9272 (Paper I) and AZD2066 (Paper II), are included in this thesis.

When the AZD9272 study was performed, there was no validated mGluR5 radioligand for PET available, thus it was explored if a \textsuperscript{11}C-labeled version of AZD9272 itself could be used as a radioligand for receptor occupancy studies. The suitability of \textsuperscript{[11]C}AZD9272 as a PET-ligand was evaluated in primate\textsuperscript{69} and human subjects (data on file). Occupancy of AZD2066 was studied using the radioligand \textsuperscript{[11]C}-ABP688, a highly selective radioligand with favorable kinetics for \textit{in vivo} imaging of mGluR5 receptors in human using PET\textsuperscript{70}.

AZD3783 (Paper III) is a high-affinity 5-HT1B receptor antagonist with potential antidepressant effects\textsuperscript{29}. The receptor occupancy of AZD3783 was investigated using the radioligand \textsuperscript{[11]C}AZ10419369 which has suitable characteristics for quantification of 5-HT1B receptor binding by PET\textsuperscript{71}.

Model implementation

Models for random effects (variability)

Random effects for inter-individual (IIV) and inter-occasion (IOV) variability were assumed to be log normally distributed according to the following:

\textit{Eq. 10}\quad p_{jk} = \theta \cdot e^{n_h + \eta_{ij}}
where $P_{jk}$ is the parameter value in the model for subject $j$ at occasion $k$, $\theta$ is the typical parameter value in the population and $\eta$s are zero-mean, normally distributed variables with standard deviation $\omega_{PIIV}$ and $\omega_{PIOV}$ which is estimated as part of the population model.

The residual error model is used to describe the difference between the model prediction based on the individual parameters ($P_{jk}$) and the observations. In the cases where radioligand concentrations were modeled directly (paper I and III), additive residual error models accounting for the correlation between the observation in the different brain regions were used, according to

Eq. 11 \[ C_{obs} = C_{ijkl} + (\varepsilon_{ijkl} + \varepsilon_{joint,ijkl}) \cdot WT \]

in which $C_{ijkl}$ is the model predicted brain concentrations of the $i$th ROI, the $j$th individual at occasion $k$ and time $l$. $C_{obs}$ are the corresponding observed concentrations. The deviations of the observations from the model predictions, the residual error, are represented by $\varepsilon_{ijkl}$ and $\varepsilon_{joint,ijkl}$, where $\varepsilon_{joint,ijkl}$ is a common residual error, accounting for the correlation between the observations in the different ROIs. The residual errors are assumed to be normally distributed with a mean of zero and variances $\sigma_i^2$ and $\sigma_{joint}^2$. In addition a weight (WT) was estimated to account for the difference in residual error at the early time points of the PET-measurement when the frame duration was less than 3 minutes.

In paper II where the modelling was based on KP$_T$, the following proportional residual error model was used

Eq. 12 \[ KP_{T,obs} = KP_{T,ijkl}(1 + \varepsilon_{ijk}) \]

In which $KP_{T,ijkl}$ is the model predicted KP$_T$ for region $i$, subject $j$ and occasion $k$, and $KP_{T,obs}$ is the corresponding observed KP$_T$. The deviations of the observations from model predictions are represented by $\varepsilon_{ijk}$. The values for $\varepsilon_{ijk}$ are assumed to be normally distributed with a mean of zero and a variance $\sigma^2$.

Paper I - AZD9272

The two tissue-compartment model (Eq. 1 and Eq. 2) was applied to model the radioligand kinetics of AZD9272. In this case the estimation of $B_{MAX}$ and $K_d$ is applicable since the tracer/radioligand and the cold compound are the same molecular species. When the PET assessment is performed after pretreatment with cold AZD9272, the free receptor concentration [$R_{Free}$] of radioligand will be reduced due to binding of unlabeled AZD9272 to the receptors. According to the law of mass action, the [$R_{Free}$] at equilibrium depends
on the non-displaceable plasma concentration in the brain ($C_{ND}$) according to the following saturation model:

Eq. 13 \[ [R_{\text{Free}}] = B_{\text{MAX}} \cdot \left(1 - \frac{C_{ND}}{C_{ND} + K_{d_{ND}}} \right) \]

Where $B_{\text{MAX}}$ corresponds to the receptor density and $K_{d_{ND}}$ is the affinity constant, i.e. the non-displaceable CNS concentration resulting in 50% occupancy. The non-displaceable concentration in the brain resulting from the oral administration of unlabeled AZD9272 was modelled according to

Eq. 14 \[ C_{ND} = C_{\text{PET}} \cdot K_{P_{ND}} \]

where $C_{\text{PET}}$ is the total (unlabeled) AZD9272 concentration in plasma and $K_{P_{ND}}$ (the brain/plasma ratio) is assumed to be the same for unlabeled and $[^{11}\text{C}]$-labeled AZD9272. The contribution of $[^{11}\text{C}]$AZD9272 to the $C_{ND}$ was thus assumed to be negligible. The plasma concentration that results in 50% occupancy ($K_{d_{PL}}$) is

Eq. 15 \[ K_{d_{PL}} = K_{d_{ND}} / K_{P_{ND}} \]

Two brain regions, ventral striatum (VST) and cerebellum (CER), the regions with the highest and lowest specific uptake, respectively, were analyzed simultaneously with two differential equations for each region describing the kinetics of brain uptake and specific binding. The extent of non-displaceable uptake ($K_{P_{ND}} = K_{1}/k_{2}$) was estimated as a parameter and assumed to be the same in both regions while the rate constant $k_{2}$ was allowed to differ. In order to assess how sensitive the estimated $K_{d_{PL}}$ was to the assumption on $K_{P_{ND}}$, an analysis was performed where the $K_{P_{ND}}$ was allowed to differ between regions by estimating a parameter corresponding to $K_{P_{ND}}$ in cerebellum relative to ventral striatum. $B_{\text{MAX}}$ for ventral striatum and $B_{\text{MAX}}$ for Cerebellum relative to ventral striatum were estimated.

**Paper II - AZD2066**

For the AZD2066 occupancy study, the analysis was based on the brain-plasma partition coefficient, $K_{P_{T}}$, derived separately from each PET-assessment. The analysis included data from 10 delineated brain regions of interest as well as the average plasma concentration of AZD2066 assessed during the PET experiment ($C_{\text{PET}}$). An NLME model was developed where this data was included in one simultaneous fit. The relationship between AZD2066 exposure and binding potential was assessed based on the following saturation function
Eq. 16  \[ BP_{ND} = BP_{BL} \cdot \left(1 - \frac{C_{PET}}{C_{PET} + Ki_{pl}}\right) \]

where \(Ki_{pl}\) is the plasma concentration corresponding to 50% occupancy, \(C_{PET}\) is the average plasma concentration of AZD2066 during the PET-experiment and \(BP_{BL}\) is the baseline \(BP_{ND}\). To reduce correlation between parameters, the model was parameterized such that \(BP_{BL}\) for caudate (\(BP_{BL,CAU}\)) was estimated as a parameter. For other regions, the \(BP_{BL}\) relative to caudate (\(BP_{REL}\)) was estimated. The \(KP_{ND}\) was assumed to be the same for all regions and the model predicted \(KP_T\) was

Eq. 17  \[ KP_T = (BP_{ND}+1) \cdot KP_{ND} \]

**Lassen method**

For comparison, occupancy was also estimated by the Lassen approach\(^{24, 31}\). With this method the difference between \(KP_T\) at the baseline PET (\(KP_{T,BL}\)) and the \(KP_T\) obtained after pretreatment (\(KP_{T,PT}\)) of the 10 regions included in the analysis was plotted versus \(KP_{T,BL}\). Linear regression was then performed to estimate the slope and intercept for all pre-treatment PET experiments. Occupancy corresponds to the slope and the intercept on the X-axis corresponds to the \(KP_{ND}\). This analysis was based on the assumption that \(KP_{ND}\) and occupancy is the same in all regions included in the analysis. The \(Ki_{pl}\) was subsequently estimated by fitting the following hyperbola to the derived occupancy values.

Eq. 18  \[ \text{Occupancy\%} = \frac{C_p \times 100}{C_p + Ki_{pl}} \]

**Simulation study**

In order to evaluate whether the results of the Lassen approach was compatible with the results of the NLME model, a simulation study was performed. Based on the final NLME model, 300 studies of identical design as the executed study, was simulated. The occupancy was subsequently estimated in each of the simulated studies with the Lassen approach as well as with the NLME model.

**Paper III - AZD3783**

In the AZD3783 study of Paper III an NLME model was developed based on the simplified reference tissue model (SRTM)\(^{17}\), which had previously been shown to be suitable for this radioligand using CER as reference region\(^{29, 71}\).
The SRTM includes the three parameters R1, k2 and BP_{ND} where k2 is the rate constant for transfer to plasma from the free and non-specific concentration in the region of interest, BP_{ND} is defined in Eq. 6 and Eq. 7 and R1 accounts for difference in equilibration rate between the target and reference regions according to

\[ R1 = \frac{k2}{k2_{REF}} \]

where k2_{REF} is the rate constant for transfer to plasma from the non-displaceable concentration in the reference region. The SRTM assumes rapid equilibrium in the reference region and the region of interest, such that specific or non-specific binding is not kinetically distinguishable. In addition, identical KP_{ND} in the reference region and the ROI is assumed. The SRTM was implemented in differential form as described previously\textsuperscript{43} according to

\[
\frac{dy}{dt} = \left( k2 - \frac{R1 \cdot k2}{1 + BP_{ND}} \right) C_{REF}(t) - \frac{k2}{(1 + BP_{ND})} \cdot y
\]

\[ C_{ROI}(t) = y + R1 \cdot C_{REF}(t) \]

where C_{ROI} is the brain concentration in the region of interest and C_{REF} is the concentration in the reference region at time t.

In the initial modelling of individual brain regions, Caudate appeared to have higher affinity (lower Ki_{PL} estimate) compared to other brain regions of interest. In order to assess whether any differences between regions in terms of KP_{ND} influenced the results, the model was extended to include the parameter ND\textsubscript{REL} according to

\[
\frac{dy}{dt} = \left( k2 \cdot ND_{REL} - \frac{R1 \cdot k2}{1 + BP_{ND}} \right) C_{REF}(t) - \frac{k2}{(1 + BP_{ND})} \cdot y
\]

\[ C_{ROI}(t) = y + R1 \cdot C_{REF}(t) \]

where the additional parameter ND\textsubscript{REL} corresponds to the non-displaceable brain-plasma partition coefficient of the ROI (KP_{ND}) relative to that in the reference region (KP_{REF}).

The analysis was based on five different ROIs with varying specific binding. In the primary analysis the cerebellum (CER) was used as reference region. A model was developed where all PET examinations and ROIs were included simultaneously and where the radioligand kinetics and the relationship between plasma concentration and saturation of specific binding were assessed simultaneously in the model.

In order to investigate the possibility of using white matter (WM) as reference region models were also fitted to data with WM as reference region.
In this case the ND\textsubscript{REL} corresponded to KP\textsubscript{ND} in WM relative to Grey matter regions. The relationship between BP\textsubscript{ND} for ROI \(i\) (BP\textsubscript{ND,i}) and AZD3783 exposure was assessed based on the saturation function described in Eq. 16. The \(k_2\) of the reference region (\(k_2\textsubscript{REF}\)) was estimated as a parameter and a separate R1 was estimated for each ROI.

Three different models with CER as reference region were applied to investigate the reason for the initial results which indicated a difference with respect to Ki in CAU.

1. CER-Base: Base model with no difference between ROIs in terms of Ki\textsubscript{PL} or KP\textsubscript{ND}.
2. CER-ND: KP\textsubscript{ND} in CAU relative to other regions (ND\textsubscript{REL}) was estimated.
3. CER-KI: Ki\textsubscript{PL} in CAU relative to other regions (Ki\textsubscript{REL}) was estimated.

Simulations

A simulation experiment was performed to investigate whether it is possible to identify and account for a different KP\textsubscript{ND} in the REF region relative to ROIs. Simulations were performed where KP\textsubscript{ND} in the reference region was 90\% of that in regions of interest (ND\textsubscript{REL}=0.9). Based on the simulated data, estimations of model parameters were performed using the simulation model where ND\textsubscript{REL} was estimated as well as with an alternative model where ND\textsubscript{REL} was fixed to 1.

Simulations were performed based on the final estimates of the WM-ND model which included four regions of interest. The Ki\textsubscript{PL} was however set to 10, 30 or 100, to mimic situations in which the highest doses in the study induces occupancy of around 45\% (Ki\textsubscript{PL}=100) up to 90\% (Ki\textsubscript{PL}=10). In order to assess whether inclusion of a ROI with low BP\textsubscript{ND} improves the precision in the Ki\textsubscript{PL}, the BP\textsubscript{ND} was either around 0.8 for all ROIs or with BP\textsubscript{ND} set to 0.2 in one of the ROIs.

Paper IVa Optimal dose selection for receptor occupancy study by PET

The relationship between drug-concentration in plasma during the PET-experiment (C\textsubscript{PET}) and KP\textsubscript{T} can be described according to the following relationship:

\[ KP_T = KP_{S0} \left(1 - \frac{C_{PET}}{Ki_{PL} + C_{PET}}\right) + KP_{ND} \]

where KP\textsubscript{S0} is the KP\textsubscript{S} in the absence of drug. The model is illustrated in Figure 2.
To estimate $K_{P0}$ and $K_{iPL}$, information on $K_{PND}$ is also needed. Information on the $K_{PND}$ can be obtained by performing the PET measurement after administration of high doses of the displacer, fully displacing the radioligand from the receptor. The $K_{PND}$ parameter can also be informed by inclusion of a reference region, or by including two or more regions with different $K_{PS}$ in the analysis, assuming that $K_{PND}$ is the same in the different regions. The need for high doses to inform $K_{PND}$ should then be reduced.

**Design situations**

The optimal design was evaluated assuming that the analysis would either be based on

- a single region with high specific binding ($K_{P0}=20$) or
- two regions that are simultaneously included, one with high ($K_{P0}=20$) and one with low specific binding ($K_{P0}=4$) or
- a reference region, assumed to be void of receptors ($K_{PS}$ fixed to zero), together with a region with high specific binding ($K_{P0}=20$).

**Assumed parameter values**

The parameter values were in the range typically seen for radioligands used to investigate receptor occupancy using PET. $K_{iPL}$ was arbitrarily set to be 1 and $K_{PND}$ was assumed to be four. The assumed parameter values are illustrated graphically using a proportional residual error ($cv=17\%$) in Figure 3. The optimal design assuming an additive error of 1.4 for the $K_{PT}$ was also
investigated. No pharmacokinetic model was included and the CPET was set equal to the dose.

![Figure 3. Illustration of the three different situations considered in the optimal design evaluation.](image)

**Study design and conditions for dose optimization**

A standard design for receptor occupancy studies including six subjects was assumed. For each subject, the design included one baseline PET-measurement and two PET-measurements after treatment with different doses of the displacing drug. The optimal pre-treatment doses were to be determined for each subject based on optimal design methodology. The doses were selected, in the range 0-8 where a dose of 8 corresponds to a receptor occupancy of 89%. In addition, to explore the impact of a reduced maximum dose, the optimal dose selection was determined in the range 0-1.5, where a dose of 1.5 corresponds to an occupancy of 60%.

The optimal designs were obtained based on either the D or the Ds optimal criterion with $K_{iPL}$ defined as the parameter of interest. The optimal designs under the conditions listed in Table 4 were determined for each of the design situations (Single region, Two regions, Reference region).

<table>
<thead>
<tr>
<th>Condition Label</th>
<th>Residual error</th>
<th>Max-dose</th>
<th>Design criterion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base case</td>
<td>Proportional</td>
<td>8</td>
<td>Ds$^1$</td>
</tr>
<tr>
<td>Additive error</td>
<td>Additive</td>
<td>8</td>
<td>Ds$^1$</td>
</tr>
<tr>
<td>D-optimal</td>
<td>Proportional</td>
<td>8</td>
<td>D</td>
</tr>
<tr>
<td>Reduced max dose</td>
<td>Proportional</td>
<td>1.5</td>
<td>Ds$^1$</td>
</tr>
</tbody>
</table>

$^1$: $K_{iPL}$ defined as the parameter of interest.
Neuropathic pain is commonly assessed by the use of an 11 point numerical rating scale (NRS) where 0 is no pain and 10 corresponds to the maximum possible pain. The present work was based on a model describing improvement from baseline at end of treatment in the NRS score. A sigmoid Emax model Eq. 23 was considered for the relationship between plasma concentrations at steady state (Css) and efficacy (E).

\[
E = E_{\text{PLACEBO}} + \frac{E_{\text{MAX}} \cdot \text{Css}^\lambda}{EC_{50}^\gamma + \text{Css}^\gamma} + \varepsilon
\]

The model included the parameters \(E_{\text{PLACEBO}}\) which is the placebo response, \(E_{\text{MAX}}\) which is the maximum effect attributable to the drug, \(EC_{50}\) which is the C\text{ss} that produces half of \(E_{\text{MAX}}\) and \(\gamma\) which is a slope factor, determining the steepness of the curve. An additive residual error (\(\varepsilon\)) was assumed. The model was reparameterized as described before\(^{58, 59}\) (Eq. 24). The parameter \(\alpha\) corresponds to \(1/EC_{50}\). The parameters \(C^*\) and \(E^*\) is a concentration effect pair on the concentration response curve, one being chosen appropriately and the other being an estimated model parameter. In this case \(E^*\) was fixed to the minimum clinically relevant efficacy and the concentration corresponding to that efficacy, \(C^*\), was to be estimated as a parameter. Designs that improves the precision of the parameter \(C^*\) will hence improve the ability to define the plasma concentration needed to achieve the minimum clinically relevant efficacy.

\[
E = E_{\text{PLACEBO}} + E^* \cdot \frac{\text{Css}^\gamma}{C^*^\gamma} \cdot \frac{1 + (\alpha \cdot C^*)^\gamma}{1 + (\alpha \cdot \text{Css}_i)^\gamma} + \varepsilon
\]

The individual plasma concentration at steady state was based on a simplified pharmacokinetic model assuming dose proportional pharmacokinetics and exponential inter-individual variability in CL/F.

**Assumed parameter values**

The \(EC_{50}\) was given the value 1. The population estimate for CL/F (\(\theta_{CL}\)) was assumed to be 1 such that for the typical individual (\(\eta_{CL}=0\)) a dose of 1 unit per day achieves a C\text{ss} of 1 and hence 50\% of the maximum efficacy. At the maximum tolerated dose (1.56), the difference in response rate was assumed to be 1.2, which was considered to be needed to have a competitive drug on the market. \(E^*\) was fixed to 1 which was considered to be the minimum clinically relevant effect as previously suggested\(^ {56}\). \(C^*\) was derived based on the assumed value for \(EC_{50}\), the efficacy at the maximum tolerated dose and the CL/F. The assumed parameter values are summarized in Table 2 and the
predicted exposure response relationship based on these parameter values is illustrated in Figure 4.

Table 2.  Assumed parameter estimates for neuropathic pain relationship

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>α(1/EC\textsubscript{50})</td>
<td>1</td>
<td>Corresponds to 1/ EC\textsubscript{50}. A value of zero corresponds to a linear relationship.</td>
</tr>
<tr>
<td>E*</td>
<td>1 Fixed</td>
<td>Minimum clinical relevant effect on the NRS-scale.</td>
</tr>
<tr>
<td>C*</td>
<td>1.04</td>
<td>Concentration inducing an efficacy of 1 (E*).</td>
</tr>
<tr>
<td>γ</td>
<td>1</td>
<td>Slope parameter assumed to be 1, but estimated.</td>
</tr>
<tr>
<td>Placebo response</td>
<td>1.2</td>
<td>Improvement on the 11-point NRS-scale in the placebo group.</td>
</tr>
<tr>
<td>σ (NRS) (SD)</td>
<td>2.1</td>
<td>Additive residual error in improvement from baseline in pain rating on the NRS-scale. Based on literature\textsuperscript{72}.</td>
</tr>
<tr>
<td>θ\textsubscript{CL}</td>
<td>1(24h\textsuperscript{-1})</td>
<td>Normalized so that a dose rate of 1 results in a Css of 1 for the typical individual (η\textsubscript{CL}=0). Fixed in optimal design evaluation, estimated in simulations.</td>
</tr>
<tr>
<td>Ω\textsubscript{CL} (CV%)</td>
<td>44%</td>
<td>Inter-individual variability in CL/F. In the normal range for a drug eliminated by metabolism. Fixed in optimal design, estimated in simulations.</td>
</tr>
</tbody>
</table>

Figure 4. Illustration of assumed relationship between plasma concentration and response as well as the variability in improvement from baseline. Circles are the simulated observations, the solid line indicates the model predicted mean treatment response.
Study design
The assumed study consisted of four arms; placebo, once daily administration of the maximum tolerated dose (1.56), and two doses that were to be optimized in the dose range 0-1.56. The sample size was based on a power calculation to detect a difference from placebo using a t-test. The number of patients needed to detect an effect at a 5% two-sided significance level and a power of 84% was 54 per treatment arm. The sample size was calculated assuming sequential hypothesis testing, starting with the highest dose which was assumed to have a treatment effect of 1.2. For convenience this sample size was used to evaluate the precision of the study designs.

Simulation studies
The precision of the concentration corresponding to the minimum clinically relevant effect can be directly obtained as the precision of C* based on the FIM. Simulation studies were however performed to derive the precision of the minimum effective dose (MED) which also is influenced by the uncertainty in CL/F. The ability to discriminate between models based on the different study designs also influences the determination of MED and was therefore accounted for in the simulation study.

The number of simulated studies was 1000 per design. For each simulated study, a model without effect, a linear model, an Emax model and a sigmoid Emax model were fitted sequentially to the simulated data. For each added parameter a likelihood ratio test (LRT) was performed at a significance level of 0.05 corresponding to a drop of 3.84 in the NONMEM objective function value (OFV). A later model was only considered if the preceding created a significant change in the OFV. The MED was then derived, for each of the simulated data-sets, based on the final estimation model.

Software
All non-linear mixed effects analyses were performed using NONMEM (versions V, VI and 7)\textsuperscript{73}. The PsN toolkit\textsuperscript{74} was used to automate procedures with NONMEM. R and the Xpose package\textsuperscript{75} implemented in R was used for goodness of fit assessment and production of graphs. The optimal design work was performed using PopED 2.13, an optimal design software for non-linear mixed effects models\textsuperscript{76}.
Results

Paper I - AZD9272

The lowest and highest radioactivity concentrations in gray matter regions of interest were seen in Cerebellum (CER) and Ventral striatum (VST) respectively. These two brain regions were considered most informative and were therefore included in the model-based analysis. The reduction in $[^{11}C]$AZD9272 concentrations between the baseline PET and after pre-treatment of 24mg AZD9272 is illustrated in Figure 5 for 10 ROIs, highlighting CER and VST.

A model was developed based on the principles of radioligand kinetics and receptor binding, which could describe this reduction. The concentration in the brain observed by the PET-system corresponds to the sum of the non-displaceable concentration, the specific receptor-bound concentration as well as a contribution from radioactivity in blood. The individual model predicted time course of concentrations in the different compartments as well as the total concentration (ipred) at baseline and after pre-treatment with 24mg AZD9272 for subject 4 is illustrated in Figure 6. The $B_{\text{MAX}}$ of CER was estimated to be 29% of VST which agreed well with literature data based on autoradiography\textsuperscript{77}. Equilibration half-life across BBB and binding to receptor was quick with a half-life of less than 2 minutes in both cases. The affinity ($K_{\text{d}_{\text{PL}}}$) was estimated to around 200 nM with high precision (relative standard error (RSE)=13%). When estimating a separate $K_{\text{PND}}$ for each of the regions, i.e. relaxing the assumption on identical $K_{\text{PND}}$, the $K_{\text{PND}}$ was estimated to be slightly lower in CER as compared to VST. The estimated affinity was slightly higher ($K_{\text{d}_{\text{PL}}}$=140nM) and the uncertainty increased (RSE= 30%) compared to the final model, illustrating the improvement in precision when assuming the same $K_{\text{PND}}$ for both ROIs. The final model included inter-individual variability on the $K_{\text{PND}}$ and inter-occasion variability on $k_2$ and $B_{\text{MAX}}$. The correlation between observations in the two regions was accounted for by inclusion of a joint residual error. The residual error was smaller in CER compared to VST probably reflecting the larger size of that ROI. The parameter estimates of the model parameters are given in Table 3.
Figure 5. Radioactive concentrations versus time in regions of interest at baseline and after pre-treatment with 24mg unlabeled AZD9272. Data from subject 4.
Table 3. Parameter estimates

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSE %</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>k2 (min⁻¹)</td>
<td>0.509</td>
<td>7.2%</td>
<td>Corresponding half-life is 1.36 minutes</td>
</tr>
<tr>
<td>KPND = K1/k2</td>
<td>0.915</td>
<td>5%</td>
<td>At equilibrium the non-displaceable concentration in CNS is 91.5% of the concentration in plasma.</td>
</tr>
<tr>
<td>RA1 = K1/K1'</td>
<td>1.14</td>
<td>2.1%</td>
<td>Ratio K1 in VST / K1 in CER</td>
</tr>
<tr>
<td>BMAX VST (nM)</td>
<td>766</td>
<td>8.8%</td>
<td></td>
</tr>
<tr>
<td>BMAX in CER relative to VST</td>
<td>0.294</td>
<td>1.7%</td>
<td>The receptor density in CER is approximately 30% of that in ventral striatum. (BMAX CER=225 nM)</td>
</tr>
<tr>
<td>KP_T at BL for VST</td>
<td>4.8</td>
<td>-</td>
<td>Total partition coefficient at BL in VST. (see eq 11)</td>
</tr>
<tr>
<td>KP_T at BL for CER</td>
<td>2.1</td>
<td>-</td>
<td>Total partition coefficient at BL in CER (see eq 11)</td>
</tr>
<tr>
<td>koff (min⁻¹)</td>
<td>0.482</td>
<td>12%</td>
<td>Corresponding half-life is 1.44 minutes</td>
</tr>
<tr>
<td>KdPl (nM)</td>
<td>196</td>
<td>13%</td>
<td>The concentration in plasma that results in 50% occupancy.</td>
</tr>
<tr>
<td>KPND IIV (CV %)</td>
<td>10</td>
<td>48%</td>
<td>Inter-individual CV in KPND.</td>
</tr>
<tr>
<td>BMAX IOV (CV %)</td>
<td>14</td>
<td>24%</td>
<td>Inter-occasion CV in BMAX</td>
</tr>
<tr>
<td>k2 IOV (CV %)</td>
<td>25</td>
<td>21%</td>
<td>Inter-occasion CV in k2</td>
</tr>
<tr>
<td>σVST (nCi/mL) (SD)</td>
<td>14</td>
<td>11%</td>
<td>Residual error for VST</td>
</tr>
<tr>
<td>σCER (nCi/mL) (SD)</td>
<td>2.26</td>
<td>119%</td>
<td>Residual error for CER</td>
</tr>
<tr>
<td>σjoint (nCi/mL)</td>
<td>9.64</td>
<td>11%</td>
<td>Standard deviation of the joint residual error accounting for the correlation between the observations in the two regions.</td>
</tr>
<tr>
<td>Weight when frame duration &lt; 3</td>
<td>1.94</td>
<td>10%</td>
<td>The residual error SD is 1.94 times higher when the frame duration is &lt;3. This is a result of increased noise and model-misspecification at early times.</td>
</tr>
</tbody>
</table>

1 RSE on random effects are expressed as 100·SE/variance.
Figure 6. Concentration in VST versus time for subject 4 at baseline and after pre-treatment with 24mg unlabeled AZD9272, including model predicted contribution from bound (specific), non-displaceable and blood concentrations of the radioligand.

Paper II - AZD2066

KP<sub>T</sub> data which was derived for each PET-measurement and 10 different ROIs were simultaneously analyzed using NLME modelling. The final model accounted for the difference in specific binding between brain regions but assumed that the non-displaceable binding and occupancy was the same across ROIs. The observed and the population predicted relationship between plasma concentration and KP<sub>T</sub> is shown in Figure 7. As seen in this figure the variability between subjects and occasions is substantial. The plot of BP<sub>ND</sub> versus exposure (Figure 8) illustrate that after accounting for variability between individuals and occasions in terms of KP<sub>ND</sub> the residual variability is small. No further improvement was seen when introducing IIV on Ki<sub>PL</sub> or BP<sub>ND</sub> in the model.

The highest uptake was seen in Caudate nucleus, hippocampus, putamen and ventral striatum which all had a BP<sub>ND</sub> of approximately 2.5 and the lowest uptake was seen in CER and pons with a BP<sub>ND</sub> of 0.75. Fixing of BP<sub>ND</sub> relative to caudate for CER to 20% based on literature data had a small impact on the Ki<sub>PL</sub> which increased from 1170 nM based on the final model to 1350 nM when fixed. The parameter estimates of the final model are shown in Table 4.
Table 4. Parameter estimates of the final model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Parameter Estimate</th>
<th>RSE%</th>
<th>BPBL,i</th>
<th>KPTBL,i</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPBL,CAU 2</td>
<td>2.46</td>
<td>23.1%</td>
<td>2.46</td>
<td>5.57</td>
<td>Binding potential relative to CAU.</td>
</tr>
<tr>
<td>BPREL,i   2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACC</td>
<td>0.88</td>
<td>3.3%</td>
<td>2.15</td>
<td>5.08</td>
<td>88%</td>
</tr>
<tr>
<td>CER</td>
<td>0.31</td>
<td>18.3%</td>
<td>0.76</td>
<td>2.84</td>
<td>31%</td>
</tr>
<tr>
<td>HIP</td>
<td>1.05</td>
<td>3.4%</td>
<td>2.58</td>
<td>5.77</td>
<td>105%</td>
</tr>
<tr>
<td>PFC</td>
<td>0.771</td>
<td>1.8%</td>
<td>1.90</td>
<td>4.66</td>
<td>77%</td>
</tr>
<tr>
<td>PONS</td>
<td>0.301</td>
<td>18.4%</td>
<td>0.74</td>
<td>2.80</td>
<td>30%</td>
</tr>
<tr>
<td>PUT</td>
<td>0.961</td>
<td>1.7%</td>
<td>2.36</td>
<td>5.42</td>
<td>96%</td>
</tr>
<tr>
<td>TC</td>
<td>0.861</td>
<td>2.7%</td>
<td>2.12</td>
<td>5.02</td>
<td>86%</td>
</tr>
<tr>
<td>THA</td>
<td>0.663</td>
<td>4.1%</td>
<td>1.63</td>
<td>4.24</td>
<td>66%</td>
</tr>
<tr>
<td>VST</td>
<td>0.991</td>
<td>1.5%</td>
<td>2.44</td>
<td>5.53</td>
<td>99%</td>
</tr>
<tr>
<td>KPND</td>
<td>1.61</td>
<td>14.1%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KiPL (nM)</td>
<td>1170</td>
<td>20.4%</td>
<td></td>
<td></td>
<td>Plasma concentration corresponding to 50% occupancy.</td>
</tr>
</tbody>
</table>

| proportional error (CV) | 5% | 7.7% |
| IOV in KPND (CV) | 9% | 12.6% |
| IIV in KPND (CV) | 23% | 26.6% |

1 The relative standard errors for omega and sigma are reported on the approximate standard deviation scale (SE/variance estimate)/2
2 Regions: CAU, caudate nucleus; ACC, anterior cingulate cortex; CER, cerebellum; HIP, hippocampus; PFC, prefrontal cortex; PUT, putamen; TC, temporal cortex; THA, thalamus; VST, ventral striatum

Analysis of the same data based on the Lassen method resulted in a similar estimate of KiPL (1165nM). The apparent variability in occupancy was however large. The result of the simulation study suggests that the variability in individual occupancy estimates seen with the Lassen approach is compatible with the random effects as implemented in the NLME model, which did not include variability in occupancy (Figure 9).
Figure 7. Population predicted $K_P_T$ (black line) and observed $K_P_T$ (points) versus $C_{PET}$ (AZD2066). Grey lines connect data from the same subject.

Figure 8. Population predicted $B_{PND}$ (black) and $B_{PND}$ based on observed $K_P_T$ and individual model prediction of $K_P_{ND}$ for each occasion (points) plotted versus $C_{PET}$. Grey lines connect data from the same subject. ($B_{PND}$ calculated as $(K_P_T/K_P_{ND} - 1)$)
Figure 9. Occupancy derived using the Lassen approach. The analysis was based on observed (left) and simulated (right) KP values. Circles represent the occupancy estimates from each pre-treatment PET, the black line is the regression curve based on these estimates. The grey line is the true occupancy used in the simulation.

Paper III - AZD3783

Cerebellum as reference region

A base model using CER as reference (CER-Base) including data from all subjects and five ROIs was developed. The model assumed the same non-displaceable binding and occupancy in all regions. Inspection of a plot of the weighted residuals (CWRES) versus time for CAU based on this analysis reveals a dose dependent pattern (top panel of Figure 10) indicating model misspecification. In order to investigate the reason for this pattern, models where CAU had a different non-displaceable binding (CER-ND) or affinity (CER-KI) compared to other ROIs were developed. The drop in OFV was 75 with CER-ND and 93 with the model CER-KI compared to CER-Base suggesting that CAU is different versus the other regions and that the difference is more likely to be related to Ki than to a difference in non-displaceable concentrations. This is further supported by plots of CWRES versus time for CAU which looks better for both CER-ND and CER-Ki compared to CER-Base and slightly better for CER-KI than for CER-ND (Figure 10). The estimated Ki for all these models were in the range 10.2-10.4 ng/mL. Based
on the CER-Ki model, the Ki_{PL} of CAU was reduced to around half of the other ROIs. The main results from these models are summarized in Table 5.

Table 5. Key parameter estimates for comparison of the models CER-Base, CER-ND and CER-Ki.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CER-Base</th>
<th>CER-ND</th>
<th>CER-Ki</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ ofv vs. base model</td>
<td>0</td>
<td>-75</td>
<td>-93</td>
</tr>
<tr>
<td>Ki_{PL} (95%CI) ng/mL</td>
<td>10.2 (7.9-13.4)</td>
<td>10.3 (8.0-13.7)</td>
<td>10.4 (8.1-13.6)</td>
</tr>
<tr>
<td>Ki_{REL} (95%CI)</td>
<td>1 FIX</td>
<td>1 FIX</td>
<td>0.55 (0.48-0.62)</td>
</tr>
<tr>
<td>ND_{REL} (95%CI)</td>
<td>1 FIX</td>
<td>0.89 (0.87-0.91)</td>
<td>1 FIX</td>
</tr>
</tbody>
</table>

Figure 10. CWRES vs time for CAU at baseline and after pre-treatment with AZD3783 based on the models CER-Base (top-panel), CER-ND (mid-panel) and CER-KI (bottom-panel). Fitted line is a loess curve.
White matter as reference region

The extended SRTM allowing the non-displaceable concentration in the reference region and ROIs to differ, opens up the possibility to use WM as reference region. In order to assess this possibility, models using WM as reference were also fitted to data. The base model (WM-Base) assumed the same non-displaceable binding in ROIs and in WM. With the alternative model (WM-ND), the non-displaceable binding in the ROI relative to WM was estimated as the parameter \( \text{ND}_{\text{REL}} \). The parameter \( \text{ND}_{\text{REL}} \) could be estimated with a high precision (RSE=3%) and the improvement over the base-model was statistically significant with a drop in OFV of 45. The \( \text{ND}_{\text{REL}} \) estimate of 0.94 was however fairly close to 1 and the resulting \( \text{Ki}_{\text{PL}} \) estimates were similar between the two models at 11.7 (RSE=19%) for WM-Base and 10.2 (RSE=24%) for WM-ND which can be compared to 10.4 for the final model using CER as reference.

Simulation study with WM as reference

In the simulation study it was possible to identify and account for a different non-displaceable uptake in the reference region relative to the ROI. The bias in the \( \text{Ki}_{\text{PL}} \) when estimating \( \text{ND}_{\text{REL}} \), was negligible in all cases, while it was 19-32% when it was fixed to 1.

If PET measurements at high occupancy were included in the study, the precision of the \( \text{Ki}_{\text{PL}} \) and \( \text{ND}_{\text{REL}} \) was improved. When \( \text{ND}_{\text{REL}} \) was fixed to 1 however, the bias increased with increasing occupancy. Inclusion of a region with low \( \text{BP}_{\text{ND}} \) in the analysis also improved the precision of \( \text{ND}_{\text{REL}} \) to some extent. The inclusion of a low-uptake region in the analysis improved the precision slightly and markedly reduced the bias in the model with \( \text{ND}_{\text{REL}} \) fixed to 1. The bias and the relative mean standard error (RMSE) of the \( \text{Ki}_{\text{PL}} \) and \( \text{ND}_{\text{REL}} \) estimate of the different simulation scenarios are shown in Figure 11.
Figure 11. Bias and RMSE of the $K_{i_{PL}}$ estimate plotted versus occupancy of the highest dose in the study based on a simulation where $K_{P_{ND}}$ in the reference region is 90% of that in regions of interest. Results shown with $N_{D_{REL}}$ estimated (left) or fixed to 1 (right). One scenario with similar $B_{P_{ND}}$ in all regions ($B_{P_{ND}}=0.8$) and one scenario where $B_{P_{ND}}$ was low ($B_{P_{ND}}=0.2$) in one of the regions of interest and 0.8 in the others.

Paper IV Optimal dose selection

Optimal dose selection for receptor occupancy

The optimal designs under the different conditions evaluated are shown in in Figure 12. As expected, for the situations with a single region and a high maximum allowed dose, all designs included the maximum dose while none of the designs with a reference region included the maximum dose. Also in the case of two regions all designs included doses at the maximum allowed level. To further elucidate the situation with two regions, an additional optimal design was derived where the $K_{P_{S0}}$ for the brain region with low specific uptake was further reduced to 1 to make the assumed data even more informative on the $K_{P_{ND}}$ parameter. The resulting optimal design did then not include any dose at the maximum allowed dose (not shown), illustrating that the larger the difference in specific uptake between regions, the less is the need for high doses.

The optimal dose-levels based on the D optimal criterion were similar to the Ds optimal dose-levels. With the Ds optimal design, more doses were however allocated to the dose-levels near the $K_{i_{PL}}$ value. With an additive error some doses were always allocated to the $K_{i_{PL}}$ level. i.e. a dose producing near 50% occupancy. With the proportional error assumed in the other
cases, the mid-dose was allocated to a higher value at 1.5 to 3 times the $K_{i_{PL}}$ value.

The FIM based predictions of the parameter precision expressed as CV% under the different conditions evaluated are shown in Figure 13. With a single brain region included in the evaluation, the precision appears acceptable if a high dose is allowed in the study. The CV in this case was in the range 18-30% depending on the conditions. With a reduced maximum dose to 1.5 times the $K_{i_{PL}}$ value (60% occupancy), the imprecision of the $K_{i_{PL}}$ parameter increased markedly (CV~80%) illustrating that including doses inducing near full occupancy is essential in the situation where the analysis is based on a single brain region.

With two regions included, with differing specific uptake, the precision was markedly improved compared to the single region case, and made it possible to obtain good precision even when the maximum allowed dose was reduced. As expected, the best precision in the $K_{i_{PL}}$ parameter was obtained when a reference region was assumed to be included. The expected precision of the $K_{i_{PL}}$ was better with the Ds optimal compared to the D optimal designs, but the difference was small.

*Figure 12.* Optimal doses. Dashed line indicates ED50 (grouped by regions included in the analysis)
Optimal dose selection for dose-finding study in neurpathic pain

The optimal doses based on the Ds-optimal criteria were 0, 0.0075, 0.73 and 1.56. Based on the D-optimal design the optimal doses were 0, 0.12, 0.63 and 1.56. The FIM based parameter precision based on the Ds-optimal design was higher compared with the D-optimal design for C* which was defined as the only parameter of interest. The precision of α and γ were higher with the D-optimal design. The precision of the placebo response and in the residual error were similar between the two designs (Table 6). Also evident is that a sigmoid Emax model will be grossly over-parameterized with very poor precision in EC\textsubscript{50} and γ even with the D-optimal design.

Table 6. Assumed parameter estimates and corresponding FIM based predicted precision based on D-optimal and Ds optimal designs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Parameter value</th>
<th>D-optimal (CV)</th>
<th>Ds-optimal (CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo response</td>
<td>1.2</td>
<td>24%</td>
<td>23%</td>
</tr>
<tr>
<td>C*</td>
<td>1.04</td>
<td>74%</td>
<td>63%</td>
</tr>
<tr>
<td>E*</td>
<td>1</td>
<td>Fixed</td>
<td>Fixed</td>
</tr>
<tr>
<td>α(1/EC\textsubscript{50})</td>
<td>1</td>
<td>472%</td>
<td>1232%</td>
</tr>
<tr>
<td>γ</td>
<td>1</td>
<td>174%</td>
<td>660%</td>
</tr>
<tr>
<td>Var (NRS) (SD)</td>
<td>2.1</td>
<td>9.7%</td>
<td>9.7%</td>
</tr>
<tr>
<td>CL/F</td>
<td>0.9 L/h</td>
<td>Fixed</td>
<td>Fixed</td>
</tr>
<tr>
<td>Ω\textsubscript{CL} (IIV, CL/F) (CV%)</td>
<td>44%</td>
<td>Fixed</td>
<td>Fixed</td>
</tr>
</tbody>
</table>
In the simulation study the probability of correct identification of the MED (defined as being within 0.5 to 2 times the correct dose) was higher with the Ds-optimal design (61%) compared to the D-optimal design (55%).
Discussion

Receptor occupancy by PET, a useful tool in drug development

The main reason for performing PET receptor occupancy studies in drug development is to facilitate translation. When developing a drug for a new target where limited clinical information is available, predictions will be based on translation from the pre-clinic to the clinic. This was the case for the first mGluR5 antagonist in a series (AZD9272) developed for neuropathic pain treatment. For the subsequent molecule in the series (AZD2066), the translation could benefit from information on the preclinical species as well as translation from the preceding candidate drug AZD9272. This was made possible by inclusion of PET-receptor occupancy studies early in these two development programs and hence allowed guidance of dosing of AZD2066, also based on the clinical results of AZD9272.

Sources of variability

Often receptor occupancy is calculated by means of a series of calculations where in the last step receptor occupancy is derived e.g. as exemplified in the AZD2066 Lassen method. With this traditional approach, all variability, including measurement error and variability between occasions will appear as variability in occupancy. Such occupancy data is easily misunderstood to represent true variability in occupancy when in fact it is a result of the combined effect of many different sources of variability. In the work presented in this thesis a more detailed analysis of the variability sources was made possible by the application of NLME analyses of data. In the papers included in the present thesis, the variability between subjects and measurement occasions was explained by variability in either non-displaceable binding or specific binding but in neither of the papers were inter-individual variability in the affinity parameter identifiable.

Comparison of the different modelling approaches.

The analyses of data were performed using three different approaches. In paper I (AZD9272), drug and radioligand were the same molecular species. To fully make use of this information, the radioligand kinetics and receptor occupancy was estimated simultaneously. Since no region, void of receptors
exists for mGluR5, arterial sampling was applied, and the model was based on arterial plasma concentrations as input function. To improve identifiability of the non-displaceable binding from the specific binding, two brain regions of interest, one with high receptor density and one with low density were included in the analysis. Population analyses has been performed previously on radioligand concentrations. This was however the first example in the literature where concentrations from two brain regions were modelled simultaneously to improve the precision of the KiPL parameter, accounting for the correlation between observations in the two regions.

In paper II (AZD2066), the drug and the radioligand were of different molecular species; hence radioligand kinetics would not be informative of the drug under development. In this case an approach where the brain/plasma partition coefficients (KP_T) for 10 different brain regions of interest were first derived. Subsequently, KP_T estimates from all brain regions, all PET-measurements and all subjects were included in one simultaneous analysis. Compared to paper I, the model was less complex and did not include differential equations. As a result, the run-times were shorter and many brain regions of interest could be included in the model. Contrary to the modeling approach applied in paper I however; any information in the radioligand kinetics on specific binding was ignored.

The simulation study suggest that both the NLME modelling and the Lassen approach can be used to obtain a population estimate of KiPL. The individual Lassen parameter estimates where however more influenced by noise, compared to the individual estimates based on the NLME approach which also provided better insight regarding variability.

In paper III (AZD37383), no arterial samples were collected, and the simplified reference tissue model (SRTM) was applied. The SRTM was also extended to allow differences in non-displaceable concentrations between brain regions. The additional parameter that quantifies any difference in non-displaceable binding is only identifiable if a simultaneous analysis including both baseline as well as pre-treatment PET measurements is performed and cannot be obtained by the use of a two stage approach as was done in paper II. While paper III (similarly as paper I) was based on modeling of TACT-data directly, it was feasible to include more brain regions in the analysis with acceptable runtimes and model-complexity since the SRTM itself contains very few parameters and runs rapidly.

Assumptions
Receptor occupancy is not directly measured and can only be estimated if assumptions related to the disposition and binding of the radioligand and the displacing drug are made. The validity of these assumptions can be supported by a good understanding of mechanisms involved and with experimental data in vitro. In addition assumptions can be tested in the framework of
modelling. With a simultaneous analysis based on all data, these tests become much more powerful as compared to tests based on each PET-measurement separately.

It is usually assumed that the non-displaceable binding or $K_{ND}$ is the same in different brain regions of interest. The impact of this assumption was tested in Papers I and III where it was shown that relaxing this assumption can have an impact on the estimated $K_{PL}$. The level of specific binding to mGluR5 receptors in cerebellum in relation to its potential use as a reference region have been discussed in the literature $^{78-80}$. If specific binding can be assumed to be negligible, analyses applying reference based methods would facilitate performance and analysis of future studies. The results of the analyses of paper I and II suggested however that cerebellum does contain receptors. The results were consistent with published results based on autoradiography$^{77}$.

Another common assumption is that the occupancy (or affinity) is the same in different brain regions$^{31}$. A possible difference between brain regions in occupancy was considered in paper III (AZD3783) and found to be more compatible with data as compared to a difference in non-displaceable concentrations.

When modelling each PET-experiment separately it is not possible to account for any changes in concentrations of the unlabeled displacer since $k_{on} \cdot R_{FREE}$ is replaced by a constant ($k_3$). The displacer plasma-concentration therefore needs to be approximated with a mean value. In paper I, when simultaneously estimating the radioligand kinetics and affinity of the displacer, this simplification was not needed and $k_{on} \cdot R_{FREE}$ was allowed to change during the 60 minute PET-measurement directly linked to the AZD9272 plasma concentrations.

Improved study design

When designing a study it is important to define the objectives precisely. At first glance, the aim of a phase IIb dose finding study and a receptor occupancy study appears to be similar. The aim in both cases is to describe exposure response as precisely as possible to improve dose selection. In paper IV it is illustrated that the aims of these two types of studies actually do differ and that this will have consequences for how the design is optimized. For the PET-study, the affinity parameter is of primary interest while for the dose-finding study it is the curve itself that matters.

For an optimal selection of doses for a PET-study it is also important to consider whether the analysis is going to be based on a single or multiple brain regions of interest and whether any brain region can be used as reference in the analysis.
Simultaneous modelling of radioligand kinetics and occupancy provides a means to avoid unnecessary simplifications or approximations and provides a powerful way to test or relax assumptions in the analysis. In the absence of a reference region, the inclusion of two brain regions (with high and low receptor density) improves the precision of the $K_{iPL}$ parameter.

A model based on $K_P T$ values as observations allows inclusion of many brain regions in the analysis. Estimation of occupancy is possible by this approach and it provided a better understanding of variability as compared to the Lassen method.

An extension to the simplified reference tissue model was developed where the assumption on identical non-displaceable binding in the brain reference region and brain regions of interest is relaxed. This allows the evaluation of whether differences between brain regions are related to affinity or to differences in non-displaceable binding. In addition estimation of receptor occupancy using white matter as reference region can be possible.

Optimal designs for maximum precision of the affinity constant are different depending on whether a single region, two regions with different receptor density or if a reference region are included in the analysis. The more informative data is on the non-displaceable binding, the less is the need for high doses fully saturating the specific binding. When optimizing a dose-finding study, the precision of a relevant part of the exposure response curve can be improved by the use of a re-parameterized model where the dose or concentration corresponding to a target effect is estimated as a parameter. A Ds optimal design provides higher precision of the relevant parameters in the model over a D optimal design.

In conclusion, this thesis presents novel non-linear mixed effects models estimating the relationship between drug exposure and receptor occupancy, providing useful translational information, allowing for a better informed drug-development.
Vid framtagande av ett nytt läkemedel vill man identifiera det dos-spann som på ett optimalt sätt balanserar de positiva hälsoeffekterna mot oönskade biverkningar. För att välja dos behöver man ha kunskap om sambandet mellan dos och dessa effekter. Ofta finns redan relevant information baserad på liknande läkemedel i människa eller på djurförsök med det potentiella nya läkemedlet. För att ta till vara denna information behöver man översätta den så att den blir användbar för det nya läkemedlet under utveckling.

För många läkemedel är bindning till receptorer en förutsättning för farmakologisk effekt. Med hjälp av en PET-kamera kan man följa radioaktivt märkta molekylers upptag i hjärnan och deras bindning till specifika receptorer. Om man från djurförsök eller från liknande molekyler i människa känner till graden av receptor bindning som krävs för optimal effekt kan man med hjälp av PET-teknik överföra den kunskapen till det nya läkemedlet. Kunskap som annars skulle vara väldigt kostsam att ta fram.

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