Modeling and Parameter Imaging of Neuroreceptor Kinetics of $[^{11}\text{C}]-\text{DASB}$ and $[^{11}\text{C}]-\text{WAY-100635}$

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Summary

English

The quantitative knowledge of serotonin system is essential to understand the mechanisms of various pathologies. Many pathologies of high social impact like depression, Alzheimer disease, Parkinson and eating disorders (anorexia/bulimia nervosa) are, in fact, related to malfunctions of the serotonin neurotransmitter at different levels. Of all serotonin receptors, two types play an important role in these pathologies. They are receptors $5-HT_{1A}$ and the serotonin transporter (SERT).

Positron emitter tracers $[^{11}\text{C}]\text{WAY-100635}$ [45, 50, 11, 53, 55, 12, 66, 61] and $[^{11}\text{C}]\text{-DASB}$ [48, 70, 69], with PET, allow the in vivo study of the serotonin receptors $5-HT_{1A}$ and SERT, respectively, and permit the quantification of many important physiological parameters like tracer distribution volume ($V_t$) and binding potential ($BP$). PET quantification can be performed at regional (ROI) and pixel level. Both PET approaches are essential to gain deep knowledge on the serotonin system. Unfortunately, their applicability on $[^{carbonyl-11}\text{C}]\text{WAY-100635}$ and $[^{11}\text{C}]\text{-DASB}$ is limited by different modeling and parameter estimation problems.

In the present study, PET quantification of the serotonin system was performed at ROI and pixel levels and some of the recent findings and issues relative to modeling and identification methods for $[^{carbonyl-11}\text{C}]\text{WAY-100635}$ and $[^{11}\text{C}]\text{-DASB}$ are presented and discussed.

In particular, the aim of this contribution with $[^{carbonyl-11}\text{C}]\text{WAY-100635}$ was to select the best compartmental model for this tracer kinetics
and to define the validity domain of the Reference Tissue models (also referred to as compartmental models without arterial input function). To do so, Spectral Analysis (SA) [4] was first applied on a healthy young subjects dataset from an Anorexia/Bulimia nervosa study acquired at the PET Center, Department of Radiology, School of Medicine, University of Pittsburgh (USA). The numbers of spectral components obtained by SA correspond to the number of tissue compartments to be used in the ROI model [8]. Based on SA results, 3 different ROI compartmental model with arterial input function and 2 compartmental models without arterial input function were put in competition and the best model for $[{\text{carbonyl}}]^{11}$CWAY-100635 kinetics was selected. The selection was based on the pattern of fit and weighted residual, on parameters' precision and on the Akaike parsimony criteria value.

In order to define the validity domain of the Reference Tissue models, a simulation study was performed where simulated healthy brain time activity curves were identified under different hypotheses. Simulation analysis indicated that it is possible to obtained unbiased estimates of parameters only when the strict hypotheses on which Reference Tissue models are based are valid.

The aim of this contribution with $[^{11}$C]-DASB was to select the best compartmental model for this tracer kinetics and to propose a robust and fast algorithm for generation of good quality parametric images. For what concerns the selection of the best compartmental model, SA was applied on an elderly healthy control subjects dataset from an Alzheimer Disease study acquired at the PET Center, Department of Radiology, School of Medicine, University of Pittsburgh (USA). Based on SA results, 6 compartmental models with arterial input function, 2 compartmental models without arterial input function and a graphical model were put in competition and the best model for $[^{11}$C]-DASB kinetics was selected.

In order, then, to select the best algorithm for the generation of good quality $[^{11}$C]-DASB parametric images, different linear algorithms were applied on the subjects dataset. The goal here was to compare parametric images obtained by Logan graphical method [27], a very fast and simple to implement method often used in literature, with the ones obtained with dif-
ferent promising approaches [9, 73, 39]. The application of these algorithms to $[^{11}C]$-DASB subjects and the use of statistical analysis allowed to detect and quantify by simulation significant differences between parametric images and the selection of the best parametric imaging algorithm.
L’analisi quantitativa del sistema serotoninergico è essenziale per comprendere i meccanismi di diverse patologie. Molte patologie di grande impatto sociale come la depressione, l’Alzheimer, il Parkinson ed i disturbi alimentari (anoressia/bulimia nervosa) sono, infatti, riconducibili a malfunzionamenti del sistema serotoninina.

Tra tutti i recettori della serotonina, due tipi giocano un ruolo fondamentale in queste patologie. Sono i recettori $5-HT_{1A}$ ed i trasportatori della serotonina (SERT). I traccianti emitenti positroni [carbonyl $^{11}$C]WAY-100635 [45, 50, 11, 53, 55, 12, 66, 61] e [$^{11}$C]-DASB [48, 70, 69], congiuntamente alla tecnologia PET, consentono di studiare in vivo i recettori $5-HT_{1A}$ e SERT, rispettivamente, e permettono la quantificazione di molti parametri fisiologici importanti come il volume di distribuzione ($V_t$) ed il potenziale di legame ($BP$). La quantificazione di un’immagine PET può essere eseguita sia a livello di regione d’interesse (ROI) che a livello di pixel. Entrambi gli approcci sono complementari al fine di ottenere una conoscenza approfondita del sistema serotoninina. Sfortunatamente, però, la loro applicabilità ad immagini PET di [carbonyl $^{11}$C]WAY-100635 e [$^{11}$C]-DASB è limitata a causa di diversi problemi di tipo modellistico e di stima parametrica.

In questo studio, la quantificazione del sistema serotoninergico tramite immagini PET è stata eseguita sia a livello di ROI che di pixel e, i problemi legati alla modellistica ed alla stima parametrica dei traccianti [carbonyl $^{11}$C]WAY-100635 e [$^{11}$C]-DASB sono presentati, risolti e discussi.

In particolare, per il [carbonyl $^{11}$C]WAY-100635, l’obiettivo di questo studio è stato la selezione del miglior modello compartimentale della sua cinetica e la definizione del dominio di validità dei modelli a Tessuto di Riferimento (detti anche modelli compartimentali senza ingresso arteriale). A questo scopo, l’Analisi Spettrale (SA) [4] è stata preliminarmente applicata ad un gruppo di soggetti giovani sani appartenenti ad uno studio su Anorexia/Bulimia nervosa eseguito presso il PET Center, Department of Radiol-
ogy, School of Medicine, University of Pittsburgh (USA). Il numero delle componenti spettrali ottenute con la SA corrisponde al numero di compartimenti tessutali che devono essere utilizzati nel modello ROI corrispondente [8]. Sulla base dei risultati della SA, 3 diversi modelli compartimentali ad ingresso arteriale e 2 modelli compartimentali senza ingresso arteriale sono stati identificati ed è stato selezionato il modello migliore per la cinetica del $[\text{carbonyl} - ^{11}\text{C}]$WAY-100635. La selezione si è basata sugli andamenti dei fit e dei residui pesati, sulla precisione dei parametri identificati e sui valori di Akaike.

Al fine di definire il dominio di validità dei modelli a tessuto di riferimento, è stato eseguito uno studio di simulazione dove curve dell'attività tessutale del tracciatore, nel cervello sano, sono state generate ed identificate secondo diverse ipotesi. Lo studio di simulazione ha indicato che è possibile ottenere stime affidabili dei parametri solamente nel caso in cui tutte le ipotesi alla base dei modelli a tessuto di riferimento siano valide.

Per il $[^{11}\text{C}]$-DASB, l'obiettivo di questo studio è stato la selezione del miglior modello compartimentale della sua cinetica e l'identificazione di un algoritmo veloce e affidabile per la generazione di immagini parametriche di buona qualità. Per quanto riguarda la selezione del miglior modello compartimentale, la SA è stata eseguita su un gruppo di soggetti anziani sani provenienti da uno studio sull'Alzheimer Disease. I dati sono stati acquisiti presso il PET Center, Department of Radiology, School of Medicine della University of Pittsburgh (USA). Sulla base dei risultati ottenuti con la SA, 6 diversi modelli compartimentali con ingresso arteriale, 2 senza ingresso arteriale ed un modello grafico, sono stati identificati, ed è stato selezionato il miglior modello per la cinetica del $[^{11}\text{C}]$-DASB. La selezione si è basata sugli andamenti dei fit e dei residui pesati, sulla precisione dei parametri identificati e sui valori di Akaike.

Infine, allo scopo di selezionare il miglior algoritmo per la generazione d'immagini parametriche di $[^{11}\text{C}]$-DASB di buona qualità, diversi metodi di stima lineare sono stati applicati al gruppo di soggetti. L'obiettivo in questo caso era quello di confrontare le immagini ottenute con il metodo grafico di Logan [27], spesso utilizzato in questo tipo di studi, con quelle ottenute da
Summary

interessanti algoritmi proposti negli ultimi anni [9, 73, 39]. L’applicazione di questi algoritmi ai soggetti di $[\text{[11C]}]$-DASB e l’impiego di tecniche di analisi statistica hanno consentito di evidenziare e quantificare, con uno studio di simulazione, differenze statistiche significative tra le immagini parametriche ottenute e di selezionare l’algoritmo di imaging parametrico migliore.
Chapter 1

Introduction

The focus of this contribution is the quantification of serotonin system by using Positron Emission Tomography (PET) images. The serotonin system is an important neurotransmitter network involved in the modulation of various physiological functions, like the perception of pain, food intake and psychological behaviors. The quantitative knowledge of this system is essential, not only to understand how the brain respond to the various stimuli, but also to understand the mechanisms of various pathologies. In fact, alterations of the serotonin system are related to pathologies of high social impact like depression, Alzheimer disease, Parkinson, sleeping and eating disorders (anorexia and bulimia nervosa), diabetes and many more.

Of all serotonin receptors, two types are particularly involved in these pathologies: they are receptors of the family 5-HT$_{1A}$ and the serotonin transporter (SERT). Receptors 5-HT$_{1A}$ are auto-receptors in the Raphe Nuclei and post-synaptic receptors in the terminal regions, and are mainly present in the limbic structures. SERT is, instead, present in the pre-synaptic cleft of the neurons that produce serotonin in its specific neuronal networks.

Positron emitter tracers [carbonyl-$^{11}$C]WAY-100635 and [$^{11}$C]-DASB with PET allow the in vivo study of the serotonin receptors 5-HT$_{1A}$ and SERT, respectively, and permit the quantification of many important physiological parameters like the tracer distribution volume ($V_t$) and the binding potential (BP).
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Positron Emission Tomography quantification can be performed at ROI levels and pixel level. ROI advantages are the good signal to noise ratio of the time activity curves to be identified with the kinetics model and the existence of well-established techniques for the identification of the number of compartments to be used in the compartmental model (I/O models or Spectral Analysis) and for the estimation of unknown parameters (Weighted non linear least squares, WNLLS). One major drawback of this approach is the loss of resolution of the original PET image.

The advantages of the pixel approach is, on the contrary, the possibility of generating parametric images without any loss of spatial resolution, and two major drawbacks are the very low signal to noise ratio of tracer activity curves and the absence of consolidated techniques for parameter identification.

Both these investigation approaches are, anyway, not only useful but absolutely complementary in order to obtain a clear picture of the serotonin physiological system.

The importance of $[\text{carbonyl}^{11}\text{C}]\text{WAY-100635}$ in the serotonin system quantification has been shown both in animal [45, 50, 11] and human [53, 55, 12, 66, 61]. However many issues are still open since their original formulation at the 1999 Workshops on $[\text{carbonyl}^{11}\text{C}]\text{WAY-100635}$ [67, 25].

In particular, the structure of the compartmental model to be used for $[\text{carbonyl}^{11}\text{C}]\text{WAY-100635}$ quantification is still debated. For instance, tracer kinetics seems to be well described by a standard Two-tissue compartment model also in regions that are known to be devoid of specific receptors (Cerebellum, for example) and that are used as reference. Some scientists [11] think of the presence of two non-specific compartments in these regions, while others [14] suggest the presence of specific uptake. It is clear that the selection of the best compartmental model for $[\text{carbonyl}^{11}\text{C}]\text{WAY-100635}$ kinetics is necessary.

The use of the so called Reference Tissue Models [2, 33] with $[\text{carbonyl}^{11}\text{C}]\text{WAY-100635}$ also needs to be deeply explored in order to determine their validity domain. Until now, in fact, these reference models have been often used in clinical studies without considering their applicability for the selected
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ligand under study and their performance.

Another issue with \([\text{carbonyl}^{11}\text{C}]\text{WAY-100635}\) is the rapid decay of \(^{11}\text{C}\), which makes it difficult to reliably tracer measure metabolite fraction at later times of PET experiments. The exponential or linear model commonly used to fit the metabolite fraction of \(^{11}\text{C}\) tracers could have an impact on the plasma input function of the compartmental model used for the tracer, affecting, therefore, parameter estimation.

The importance of \([^{11}\text{C}]-\text{DASB}\) with PET for the study of SERT system has been shown in the last few years [48, 70, 69]. Again, research has been slowed by the lack of a validated mathematical model and by several parametric imaging issues.

For what concerns model issues, scientists still debate on the best compartmental model for \([^{11}\text{C}]-\text{DASB}\) kinetics [68], that in literature is found to be described by One- and Two-Tissue compartment models.

For what concerns imaging issues, many efforts have been recently put in determining robust and fast algorithms for the generation of good quality parametric images representing both spatial distribution and quantification of physiological parameters of tracer kinetics [73, 71, 74, 58]. In fact, parametric imaging is highly desirable as it would allow pixel-based statistical analysis across brain volumes, but the inherent high noise level of dynamic PET require the implementation of different than the standard non linear parameter estimation strategies.

The aim of this contribution with \([\text{carbonyl}^{11}\text{C}]\text{WAY-100635}\) is to select the best compartmental model for this tracer kinetics and to define the validity domain of the Reference Tissue models.

For what concerns the selection of the best compartmental model of \([\text{carbonyl}^{11}\text{C}]\text{WAY-100635}\) kinetics, Spectral Analysis (SA) [4] is applied on a 9 healthy young subjects dataset from an Anoressia/Bulimia nervosa study acquired at the PET Center, Department of Radiology, School of Medicine, University of Pittsburgh (USA). The number of spectral components obtained by SA correspond to the number of tissue compartment to be used in the ROI model [8]. The possibility that the number of spectral com-
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ponents given by SA results can change, depending on a particular tracer metabolite fraction model, is also explored. To do this, SA results obtained by both exponential and linear modeling of the metabolite fraction of $[\text{carbonyl}-^{11}\text{C}]\text{WAY-100635}$ are compared. Based on SA results, 4 different ROI compartmental model with arterial input function and 2 compartmental models without arterial input function are put in competition and the selection of the one that best describes $[\text{carbonyl}-^{11}\text{C}]\text{WAY-100635}$ kinetics is based on the pattern of fit and weighted residual, on parameters’ precision and on the Akaike parsimony criteria value.

In order to define the validity domain of the Reference Tissue models, a simulation study is performed. Simulated healthy brain time activity curves are identified under different hypotheses and bias of parameters from the true values is calculated. Tested hypothesis are the one on which Reference Tissue models are based: they concerns the reference region model (modeled by One- or Two tissue compartments), the presence or absence of vasculature volume within the ROI and the difference on the non specifically bound volumes in the reference and region of interest.

The aim of this contribution with $[^{11}\text{C}]-\text{DASB}$ is to select the best compartmental model for this tracer kinetics and to propose a robust and fast algorithm for generation of good quality parametric images.

For what concerns the selection of the best compartmental model, SA is applied on a 6 elderly healthy control subjects dataset from an Alzheimer Disease study acquired at the PET Center, Department of Radiology, School of Medicine, University of Pittsburgh (USA). Based on SA results, 6 compartmental models with arterial input function, 2 compartmental models without arterial input function and a graphical model are put in competition and the selection of the one that best describes $[^{11}\text{C}]-\text{DASB}$ kinetics is based on the pattern of fit and weighted residual, on parameters’ precision and on Akaike parsimony criteria value.

In order, then, to select the best algorithm for the generation of good quality $[^{11}\text{C}]-\text{DASB}$ parametric images, different linear algorithms are applied on the 6 subjects dataset. The goal here is to compare parametric
images obtained by Logan graphical method [27], a very fast and simple to implement method that is often used in literature, with the ones obtained with a Generalized Least Squares approach [9] and with Ridge Regression [73]. Other parametric imaging algorithms exists for PET parametric imaging [49, 58] but since their performance can be negatively affected by poor choices of a priori settings [60] they are not considered in this study.

The application of the above mentioned algorithms to the 6 $^{11}$C-DASB subjects and the use of statistical analysis allows to detect significant differences between the parametric images. In order to quantify these differences, a simulation study is performed where 3 planes of synthetic brain time activity including important brain structures for $^{11}$C-DASB are generated, and the bias between true parametric images and the ones obtained with the above mentioned approaches is calculated. In the simulation study, besides Logan, Generalized Least Squares and Ridge Regression methods, which require the knowledge of the arterial tracer concentration, 2 popular Reference Tissue approaches are also considered. They are the very frequently used Simplified Reference Tissue method proposed by Gunn et al. [59] (Basis Function method) and the recent Multilinear Reference Tissue method proposed by Ichise et al. [38].

The thesis is articulated as follows:

Chapter 2 describes the main events underlying the biosynthesis and metabolism of serotonin and serotonin receptors with particular emphasis given to receptors 5-HT$_1$A and SERT. Then, general considerations on tracers [carbonyl$^{11}$C]WAY-100635 and $^{11}$C-DASB synthesis and biodistribution follow. Finally, chapter 2 presents a brief section on neuropsychopharmacology.

Chapter 3 presents details about both $^{11}$C-DASB and [carbonyl$^{11}$C]WAY-100635 experimental protocols and acquired dataset.

Chapter 4 describes ROI models used to analyse both dataset. First, SA as implemented by Bertoldo et al. [4], compartmental models with arterial input function (Three-, Two- and One-Tissue compartment models), standard Reference Tissue models (Full and Simplified) and Logan Graphical
1. Introduction

Analysis [27] are presented. Details about estimated parameters and identification process are also reported. After this introductory section, $^{11}$C]-DASB ROI results on 6 elderly healthy controls are presented. A brief discussion of results follows. Then, $[^{11}\text{C}]$ WAY-100635 results on 9 young healthy controls are reported. In this section is first presented the study on the metabolite fraction modeling; then, results on the compartmental modeling are reported and, finally, the simulation analysis on the Reference Tissue models is presented. A discussion of results follows.

Chapter 5 is relative to the pixel analysis performed on 6 $^{11}$C]-DASB subjects. First, Generalized Least Squares [9], Ridge Regression [73], Multilinear [39] and Basis Function [59] methods are presented. Then, results relative to both the real dataset and the simulation study are reported. A discussion follows.

Chapter 6 discusses results obtained in this study about $[^{11}\text{C}]$ WAY-100635 and $^{11}$C]-DASB quantification as well as emerged open questions and future direction of research.
Chapter 2

The Serotonin System

Of all the neurotransmitters, serotonin remains historically the most intimately involved in neuropsychopharmacology, and when 5-HT was found within the mammalian CNS, the theory arose that various forms of mental illness could be due to biochemical abnormalities in its synthesis [13, 15, 19]. As we see today, many of these ideas and theories are still maintained, although now there is much more ample evidence with which to evaluate them. In this chapter, main events underlying serotonin biosynthesis as well as its receptors and PET radioligand employed in this study are presented and discussed.

2.1 Biosynthesis and Metabolism of Serotonin

Serotonin is found in many cells that are not neurons, such as platelets, mast cells, and the enterochromaffin cells mentioned above. In fact, only about 1%-2% of the serotonin in the whole body is found in the brain. Nevertheless, because 5-HT cannot cross the Blood-Brain barrier (BBB), it is clear the brain cells must synthesize it their own.

For brain cells (see Fig.2.1), the first important step for serotonin biosynthesis is the uptake of the amino acid tryptophan which arises primarily from the diet.

The second step in the synthetic pathway is hydroxylation of tryptophan
2. The Serotonin System

Figure 2.1: The metabolic pathway for serotonin synthesis

at the 5 position to form 5-hydroxytryptophan (5-HTP). The enzyme responsible for this reaction, tryptophan hydroxylase, occurs in low concentrations in most tissues, including the brain.

Once synthesized from tryptophan, 5-HTP is almost immediately decarboxylated to yield serotonin, which is released during neuronal activation in the synaptic cleft.

Serotonin is converted into inactive molecules by bio transformations (Fig.2.2) [22]. The route of its continued metabolism is deamination by monoamine oxidase. The product of this reaction, 5-hydroxyindoleacetaldehyde, can be further oxidized to 5-hydroxyindoleacetic acid (5-HIAA) or reduced to 5-hydroxytryptophol.

Another major mechanism for the termination of the action of synaptic serotonin is reuptake across the pre-synaptic membrane. Serotonin nerve terminals possess high-affinity serotonin uptake sites that play an important role in terminating transmitter action and in maintaining transmitter homeostasis (SERT). Reuptake is an energy dependent process accomplished by a plasma membrane carrier that is capable of transporting serotonin in either direction, depending on the concentration gradient. Sodium/potassium ATPases use energy from ATP hydrolysis to create a concentration gradi-
2. The Serotonin System

Figure 2.2: Main pathway of serotonin catabolism

ent of ions across the pre-synaptic membrane that drives the opening of the transporter and co-transport of sodium and chloride ions and 5-HT from the synaptic cleft. Potassium ions binding to the transporter enable it to return to the outward position. Release of the potassium ions into the synaptic cleft equilibrates the ionic gradient across the pre-synaptic membrane. The 5-HT re-uptake transporter is then available to bind another 5-HT molecule for re-uptake. Although the involvement of transporters in serotonin clearance has been appreciated for several decades, progress in understanding transporter structure and regulation has been slow and is at the moment the target of many research studies.

Finally, a very important organ for the study of the serotonin system is the pineal body, a tiny gland contained within connective tissue extensions of the dorsal surface of the thalamus. This organ is important for two reasons: first, it contains all enzymes required for the synthesis of serotonin plus two enzymes for further processing serotonin, which are not so pronounced in
2. The Serotonin System

other organs (the pineal contains more than 50 times as much 5-TH/gr as the whole brain); second, the metabolic activity of the pineal 5-HT enzymes can be controlled by numerous external factors, including the neural activity of the sympathetic nervous system operating through release of norepinephrine. In this gland, serotonin is transformed into melatonin. This transformation is not regarded to as a degradation pathway because melatonin is also an active molecule. Two steps are involved in melatonin production (Fig. 2.3): the first is the acetylation of the amine group by N-acetyl transferase leading to N-acetyl-serotonin. The second is the methylation of the OH group by 5-hydroxyindole-O-methyltransferase catalyzing the transfer of a methyl group from S-adenosyl-methionine to obtain acetyl-5-methoxytryptamine or melatonin. The concentration of melatonin in the pineal gland presents circadian variations: it follows the variations of N-acetyl transferase activity, increasing during the night and decreasing during the day, darkness and light playing a regulatory role via catecholamines. Light inhibits melatonin biosynthesis.

Figure 2.3: Biosynthesis of melatonin
2. The Serotonin System

Figure 2.4: Schematic diagram illustrating the distribution of the main serotonin-containing pathways in the rat central nervous system.

2.2 Localizing Brain Serotonin to Brain Cells

Although most early neurochemical pharmacology assumed the brain 5-HT was a neurotransmitter, more than 10 years elapsed before it could be established with certainty that 5-HT in the brain is actually contained within specific nerve circuits. Serotonin containing neurons are known to be restricted to clusters of cells lying in or near the midline or raphe regions of the pons and upper brain stem (Fig. 2.4). In addition to the nine 5-HT nuclei \((B_1-B_9)\), chemical localization of 5-HT has also detected reactive cells in the area postrema and in the caudal locus ceruleus, as well as in and around the interpeduncular nucleus. The more caudal groups project largely to the medulla and spinal cord. The more rostral 5-HT cell groups are thought to provide the extensive 5-HT innervation of the telencephalon (i.e. limbic system, cerebral cortex, basal ganglia) and diencephalon (i.e. thalamus and hypothalamus). The intermediate groups may project into both ascending and descending groups. Immunocytochemical studies also revealed a far more extensive innervation of cerebral cortex, which is quite patternless in general.
2. The Serotonin System

The raphe neurons appear to innervate overlapping terminal fields. Exceptions to this generalization is the $B_8$ group (raphe medius) that appears to furnish a very large component of 5-HT innervation of the limbic system, while $B_7$ (or dorsal raphe) projects with greater density to the neostriatum, cerebral and cerebellar cortices, and thalamus.

The application of sensitive techniques has revealed that the cerebral cortex in many mammals is innervated by two morphologically distinct types of 5-HT axon terminals. "Fine axons" with small varicosities originate from the dorsal raphe nuclei, and "beaded axons" with large spherical varicosity arise from the median raphe nuclei. These two types of 5-HT containing axons have different regional and laminar distributions, but, at present, the functional role played by the fine and beaded axons systems and whether the functions are distinct or similar remain uncertain.

2.3 5-HT Receptors

The advent of receptor-binding studies in the 1970s revealed the existence of multiple receptors for serotonin in the CNS. At present, seven subtypes of serotonin receptors have been defined and characterized in brain tissue, based on radioligand binding studies. Serotonin receptors were divided into several subclasses based on functional similarities and their characteristics are listed in Table 2.1.

Particularly interesting for the study of the serotonin system is subtype 5-HT$_{1A}$ [72]. 5-HT$_{1A}$ receptors are distributed largely throughout the CNS. In the raphe nuclei, they are somatodendritic and act as autoreceptors to inhibit cell firing; postsynaptic 5-HT$_{1A}$ receptors are present in a number of limbic structures, particularly the hippocampus. It has been established, also, that activation of postsynaptic 5-HT$_{1A}$ receptors induces a behavioral syndrome, characterized by flat body posture, reciprocal forepaw treading and head weaving. A decrease in blood pressure and heart rate and increased locomotor responses can be induced by central 5-HT$_{1A}$ receptor activation, whilst fluoxetine-induced penile erections can be markedly potentiated by combined 5-HT/5-HT$_{1A/1B}$ receptor blockade. The proposed role of 5-HT$_{1A}$ receptors in
## Table 2.1: Mammalian 5-HT receptors subtypes

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<th>5-HT₃</th>
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<td>Gq/11</td>
<td>ion channel</td>
</tr>
<tr>
<td>Agonists</td>
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<td>α-methyl-5-HT</td>
<td>2-methyl-5-HT</td>
</tr>
<tr>
<td>Antagonists</td>
<td>-</td>
<td>Katanserin (5-HT₂₄)</td>
<td>odansetron</td>
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<td>-</td>
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<tr>
<td>Effector</td>
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<td>?</td>
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<td>Gs</td>
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2. The Serotonin System
modulating anxiety-related behaviors is supported by many studies [34, 30].

2.4 Neuropsychopharmacology

Serotonin plays an important role in the regulation of mood, sleep, emesis (vomiting), sexuality and appetite and in many disorders, notably as part of the biochemistry of depression, migraine, bipolar disorder and anxiety [3]. Technical advancements in recent years have allowed progress toward the understanding of this system and how drugs can be made to affect it.

A variety of psychiatric medications affect serotonin levels, including the monoamine oxidase inhibitors (MAOIs), tricyclic antidepressants (TCAs), atypical antipsychotics, and the selective serotonin reuptake inhibitors (SSRIs).

The MAOIs prevent the breakdown of monoamine neurotransmitters (including serotonin), and therefore increase concentrations of the neurotransmitter in the brain. MAOI therapy is associated with many adverse drug reactions, and patients are at risk of hypertensive crisis triggered by foods with high tyramine-content and certain drugs.

Medications that inhibit this re-uptake of serotonin are TCAs and SSRIs. In particular, TCAs inhibit the reuptake of both serotonin and norepinephrine while SSRIs act only on serotonin reuptake and have fewer (though still numerous) side effects and fewer interactions with other drugs.

Since SSRIs affect only the reuptake pumps responsible for serotonin, as opposed to earlier antidepressants, which affect other monoamine neurotransmitters as well, they lack some of the side effects of the more general drugs. SSRIs have become, for these reasons, very popular in the last years. Usually, several weeks of continuous SSRI use are necessary for the antidepressant effects to become fully manifested. Pharmacologically, this delay is due to a side-effect of the initially high levels of serotonin within the synaptic cleft: high serotonin levels will not only activate the postsynaptic receptors, but also flood the autoreceptors of the presynaptic cell, triggering a throttling of serotonin production. The resulting serotonin deficiency persists for some time, as the transporter inhibition occurs downstream to the cause of
the deficiency, and is therefore not able to counterbalance it. Consequently, during SSRI therapy, the body must first adapt to the high levels of serotonin within the synaptic cleft by downregulating the sensitivity of the autoreceptors, which can take up to 3 weeks. To expedite the onset of the antidepressant effect, bifunctional SSRIs are currently under development, which will additionally occupy the autoreceptors, and thus deactivate the serotonin production throttling mechanism. Serotonin is also involved in regulation of carbohydrate metabolism. Few analysis of the role of SSRIs in treating depression cover the effects on carbohydrate metabolism from intervening in serotonin handling by the body. Also, SSRIs may protect against neurotoxicity caused by other compounds (for instance MDMA and Fenfluramine) as well as from depression itself.

There appears to be no significant difference in effectiveness between SSRIs and tricyclic antidepressants, which were the most commonly used class of antidepressants before the development of SSRIs [24]. However, SSRIs have the important advantage that their toxic dose is high. SSRIs are not addictive in the conventional medical use of the word (i.e. animals given free access to the drug do not actively seek it out and do not seek to increase the dose), but suddenly discontinuing their use is known to produce both somatic and psychological withdrawal symptoms, a phenomenon known as "SSRI discontinuation syndrome" [32].

2.5 PET Tracers

2.5.1 \([^{11}C]\)-DASB

The first PET radiotracer successfully developed to image SERT density in humans was \([^{11}C]\)-McN5652, which has been used in several clinical studies of patients with mood disorders [23], obsessive compulsive disorders [16] and ecstasy abuse [46]. However, \([^{11}C]\)-McN5652 was not without limitations and it was soon replaced by \([^{11}C]\)-DASB, a new PET radiotracer developed by Wilson and coworkers at the University of Toronto, Canada. \([^{11}C]\)-McN5652 non specific binding was, in fact, relatively high, precluding the reliable quan-
2. The Serotonin System

Figure 2.5: Time activity curves of $[^{11}C]$-DASB. Regions displayed include midbrain (black circle), thalamus (black square), striatum (triangle), medial temporal lobe (circle), anterior cingulate cortex (square), and cerebellum (black triangle). Points are measured activities in ROIs [70].

Identification of SERT in regions of moderate-to-low SERT density. Moreover, brain uptake of this radiotracer was protracted, so that long imaging sessions were required to derive accurate binding parameters.

With respect to $[^{11}C]$-McN5652, $[^{11}C]$-DASB is an imaging agent with higher specific activity and faster metabolism. As $[^{11}C]$-McN5652, after an initial rapid distribution phase, plasma activity stabilizes at relatively constant level and the activity concentrates in regions with high SERT densities (midbrain, thalamus and striatum). Intermediate activity are seen in amygdala, hippocampus, cingulate and parahippocampal gyrus, while low levels are observed in the neocortex and cerebellum (see Fig. 2.5). Differences between the two tracers are found in mean regional peak uptake time that is $42\pm15$ min for $[^{11}C]$-DASB, while it is $61\pm23$ min for $[^{11}C]$-McN5652, and in the regional values of distribution volumes. Differences are, in fact, observed between tracers and between brain regions (See Fig. 2.6). In particular, the distribution volume of $[^{11}C]$-DASB in cerebellum is significantly lower than $[^{11}C]$-McN5652 [70].

$[^{11}C]$-DASB image provides improved contrast and finer details and even
2. The Serotonin System

Figure 2.6: MR images (left) and coregistered PET images acquired from 40 to 90 min after injection of 659 MBq $[^{11}\text{C}]$-DASB (center) and 599 MBq $[^{11}\text{C}]$-McN5652 (right) in 33-y-old healthy male volunteer. Activity was normalized to injected dose and color coded using identical scale. (Top row) Coronal plane illustrates ventrodorsal gradient of SERT in striatum. (Middle row) Sagittal plane close to midline shows accumulation of activity in thalamus and caudate. Image also illustrates low level of activity in cerebellum and small difference in uptake between cerebellum and neocortical regions. (Bottom row) Transaxial plane at level of midbrain. Note very high activity concentration in dorsal raphe on $[^{11}\text{C}]$-DASB scan, just ventral to fourth ventricle. Concentration of activity in amygdala is also noticeable on $[^{11}\text{C}]$-DASB image [70].
2. The Serotonin System

though its faster kinetics do not necessarily translate in shorter minimal scan duration required to derive time-dependent estimates of volume of distribution (around 90 min), its characteristics make it the tracer of choice for the quantification of SERT, also in regions of moderate-to-low SERT density [70].

First quantitative efforts with this ligand were made by Ginovart and collaborators and the suitability of $^{11}$C-DASB for research on the SERT using PET was supported by the observation that tissue data could be described using a kinetic analysis and that simplified quantitative methods, using the cerebellum as reference, provided reliable estimates of SERT binding parameters. In particular, Ginovart et al. [48] published a study where different modeling strategies for quantification of $^{11}$C-DASB binding were evaluated in five healthy humans. Kinetic analyses were performed with compartmental models characterized by one and two tissue compartments. Time activity curves resulted well described by a One-tissue compartment model for all considered regions, while a Two-tissue compartment model with four parameters failed to converge reliably. Reliable fits of the data were obtained only if no more than three parameters were allowed to vary. However, even then, rate constants relative to tracer transport into the specific compartment were estimated with poor precision and only their ratio was stable. Goodness of fit was not improved by using a Two-tissue compartment model as compared with a One-tissue compartment model. Minimal study duration was also evaluated and estimated to about 80 min to obtain stable estimates of the binding potential. For routine use, several simplified methods using the cerebellum as a reference region were also evaluated. They all gave binding potential values consistent with those obtained with the Two-tissue compartment model.

2.5.2 $[^{11}\text{C}]\text{WAY-100635}$

Many radioligands were proposed for the visualization of the serotonin receptors in the human brain using PET. More than 20 compounds have been labelled with carbon-11 (half-life 20 min), fluorine-18 (half-life 109.8 min) or
2. The Serotonin System

iodine-123 (half-life 13.2 h) [26]. Between them, some are structural analogues of the antagonist WAY-100635.

WAY-100635 is a potent and selective 5-HT\textsubscript{1A} receptor antagonist which was developed by Wyeth-Ayerst Research. The PET Group of Hammersmith Hospital in London published the first image of 5-HT\textsubscript{1A} receptors in human brain, using \([O − methyl−11C]WAY-100635\) in 1995 [53]. The distribution radioactivity in the images corresponded to the distribution of 5-HT\textsubscript{1A} receptors, but although preliminary results were highly promising, the analysis of labelled metabolites in plasma indicated that \([O − methyl−11C]WAY-100635\) was not indicated for PET studies in the CNS because a lipophilic metabolite \((\[O − methyl−11C]WAY-100634)\) could enter the brain more easily than \([O − methyl−11C]WAY-100635\), and radioligand activity probably displayed both receptor-mediated 5-HT\textsubscript{1A} and non-specific binding. The presence of \([O − methyl−11C]WAY-100634\) seriously complicated attempts to quantify cerebral 5-HT\textsubscript{1A} receptors [51].

Because of the formation of \([O − methyl−11C]WAY-100634\), Pike and collaborators decided to radiolabel WAY-100635 in the carbonyl position. Metabolism to WAY-100634 would then result in loss of the label as cyclohexanecarboxylic acid and radiolabelled WAY-100634 would not interfere with the tomographic measurements.

The Hammersmith Group published the first results with \([\textit{carbonyl−11C}]WAY-100635\) [54]. Radioactivity in human cerebellar, a region devoid of specific receptors, was much lower after injection of \([\textit{carbonyl−11C}]WAY-100635\) than \([O − methyl−11C]WAY-100635\), indicating a lower level of non-specific binding. HPLC analysis of radioactivity in humans plasma after injection of \([\textit{carbonyl−11C}]WAY-100635\) confirmed the presence of \([11C]\text{cyclohexanecarboxylic}\) acid and some unidentified metabolites derived from further degradation of \([11C]\text{cyclohexanecarboxylic}\) which, anyway, did not cross the blood brain barrier.

Radioligand \([\textit{carbonyl−11C}]WAY-100635\) is characterized by rapid metabolism in human plasma and its activity concentrates in neocortical and limbic areas, with the highest uptake in temporal cortex and medial temporal structures (see Fig. 2.7). Very low activity levels are measured in the
Figure 2.7: Brain distribution of $[\text{carbonyl-}^{11}\text{C}]$WAY-100635 in a human volunteer. (top row) PET: Transaxial, coronal, and sagittal views acquired over 60 minutes and starting 40 minutes after the injection of 12 mCi of $[\text{carbonyl-}^{11}\text{C}]$WAY-100635 in a 30 year old male. (lower row) MRI: SPGR acquisitions in the corresponding planes. The coronal section is at the level of the anterior hippocampus. The sagittal section was taken at midline to display activity corresponding to the 5-HT$_{1A}$ somatodendritic receptors in the dorsal raphe nuclei in the pons, at the level of the floor of the 4th ventricle [61].
2. The Serotonin System

striatum, thalamus, and cerebellum. An activity concentration is also visualized in midbrain and pons at the level of the floor of the fourth ventricle, corresponding to the location of the dorsal and median raphe nuclei. Uptake peaks within 10 to 15 minutes and is followed by an appreciable washout. Overall, the brain uptake is low [61].

The success of \([\text{carbonyl}^{11}\text{C}]\text{WAY-100635}\) as a radioligand is reflected in the fact that the European Community sponsored two workshops on serotonin 5-HT\(_{1A}\) receptor imaging in the human brain with PET, for the purpose of the standardization and dissemination of the methodology [67, 25]. Many quantitative efforts were made with this ligand since those workshops, and the one that certainly needs to be mentioned was published by Parsley and collaborators in 2000 [61]. The goal of the study was to evaluate methods to derive 5-HT\(_{1A}\) receptor parameters in the human brain with PET. Five healthy volunteers were studied twice and three methods of analysis were used to derive the binding potential and the specific to nonspecific equilibrium partition coefficient. Two of these three methods, kinetic analysis based on a Two-tissue compartment model and graphical analysis, used the arterial input function to derive the unknown parameters. The third method, instead, derived the input function from uptake data of a region of reference, the cerebellum, and provided only the specific to nonspecific equilibrium partition coefficient. All methods provided estimates of regional 5-HT\(_{1A}\) receptor parameters that were consistent with the known distribution of 5-HT\(_{1A}\) receptors in the human brain. Compared with kinetic binding potential, graphical analysis slightly underestimated, and this phenomenon was mostly apparent in small size-high noise regions. Compared with kinetic specific to nonspecific equilibrium partition coefficient, the reference tissue method underestimated and the underestimation was apparent primarily in regions with high receptor density. Derivation of binding potential by both kinetic and graphical analysis was highly reliable while reliability of specific to nonspecific equilibrium partition coefficient resulted lower. Parsley \textit{et al.} [61] concluded that derivation of the binding potential by kinetic analysis using the arterial input function appeared as the method of choice because of its higher test-retest reproducibility, lower vulnerability to experimental
2. The Serotonin System

noise and absence of bias.
Chapter 3

$[^{11}\text{C}]-\text{DASB}$ and
$[^{carbonyl}^{11}\text{C}]\text{WAY}-100635$: Protocol, Subjects and Data

This chapter presents PET acquisition protocols, subjects and data.

3.1 $[^{11}\text{C}]-\text{DASB}$

3.1.1 Subjects and Radioligand

PET experiments were performed at the PET Center, Department of Radiology, School of Medicine, University of Pittsburgh (USA). Six elderly healthy controls (4 women and 2 men) from an Alzheimer Disease project were scanned. Their mean age was 60±13 years (see Table 3.1).

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</table>

Table 3.1: Age and gender of elderly healthy controls.
3. $[^{11}\text{C}]-\text{DASB}$ and $[^{11}\text{C}]\text{WAY}-100635$: Protocol, Subjects and Data

Typical radioligand time activity curves in brain tissue and plasma are illustrated below (Fig. 3.1 and 3.2).

![Figure 3.1: Typical $[^{11}\text{C}]-\text{DASB}$ time activity curves](image1)

![Figure 3.2: Typical uncorrected and corrected plasma time activity curve](image2)

This study was conducted according to local institutional review board regulations, and all participating subjects gave written informed consent.

All subjects underwent magnetic resonance (MR) imaging prior to the PET scan on a Signa 1.5 Tesla scanner (GE Medical Systems, Milwaukee, WI). The MR data were resliced to match the spatial orientation of the PET.
image data, based upon previously published methods [47]. All subjects were scanned on a Siemens ECAT HR+ PET scanner (CTI PET systems, Knoxville, TN) in three-dimensional (3D) imaging mode. The HR+ acquires 63 continuous slices over a 152-mm axial field of view. Subjects were positioned with the head oriented parallel to the canthomeatal line. A softened thermoplastic mold with generous holes for the eyes, nose, and ears was fitted closely around the head and attached to a headholder to minimize subject motion.

Prior to radiotracer injection, a 5 ml sample of arterial blood was collected and used to assess the level of $^{11}$C-DASB binding to plasma proteins. Immediately following bolus intravenous injection of $\sim 10$ mCi high-specific activity of $^{11}$C-DASB, dynamic emission scanning with arterial blood sampling (input function) was performed over 90 min. The arterial input function was determined from 35 0.5 ml hand-drawn blood samples collected over the scanning interval (1x15 sec., 8x30 sec., 10x1min., 9x5min. and 3x10min.). Blood samples were centrifuged and the plasma radioactivity concentration measured. Additionally, 3 ml 8 blood samples were acquired after $^{11}$C-DASB injection throughout the study (0, 2, 10, 20, 30, 45, 65, 85 min) and used to determine the fraction of unmetabolized $^{11}$C-DASB (of total plasma radioactivity concentration) using high-performance liquid chromatography (HPLC). Plasma data were corrected for the presence of radiolabeled metabolites of $^{11}$C-DASB using the HPLC data and input functions were fitted to a sum of exponentials plus a constant term, when necessary. PET data were corrected for radioactive decay and scatter. Image reconstruction was performed using filtered back-projection (Hann filter); the final reconstructed image resolution was 6.5-7.0 mm. A typical reconstructed $^{11}$C-DASB image is given in Fig. 3.3. The scans were visually inspected for head motion and a postprocessing correction was performed. Head motion was determined by overlaying an MR-based brain outline on each frame of the PET study. Regions of interest (ROI) were hand drawn on the coregistered MR images and applied to the dynamic PET data to generate time activity curves. The following ROIs were selected: Amygdala (AMY), Hippocampus (HIP), Dorsal Raphe (DRN), Mesial temporal Cortex (MTC), Cerebellum (CER) and
Figure 3.3: Typical PET image of $[^{11}\text{C}]-\text{DASB}$ binding of the same middle brain slice (left: time frame number 4; right: time frame number 35).

Thalamus (THL). ROI sampling of the cerebellum was also performed, and this was used as the reference region because of the low concentration of SERT receptors. The cerebellar reference region (Ref) data were assumed to be representative of the free and nonspecifically bound radioactivity concentrations, in all regions [56].

3.2 $[^{carbonyl-11}\text{C}]\text{WAY-100635}$

3.2.1 Subjects and Radioligand

PET experiments were performed at the PET Center, Department of Radiology, School of Medicine, University of Pittsburgh (USA). This study was conducted according to local institutional review board regulations, and all participating subjects gave written informed consent. Nine healthy young women from a project on Anorexia and Bulimia nervosa were scanned. Their mean age was $23\pm7$ years (see Table 3.2).
3. $[^{11}\text{C}]-\text{DASB}$ and $[^{carbonyl-11}\text{C}]\text{WAY-100635}$: Protocol, Subjects and Data

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Table 3.2: Age and gender of $[^{carbonyl-11}\text{C}]\text{WAY-100635}$ healthy controls

Typical radioligand time activity curves in brain tissue and plasma are illustrated below (Fig. 3.4 and 3.5):

![Figure 3.4: Typical $[^{carbonyl-11}\text{C}]\text{WAY-100635}$ time activity curves](image)

All subjects underwent magnetic resonance (MR) imaging prior to the PET scan on a Signa 1.5 Tesla scanner (GE Medical Systems, Milwaukee, WI). The MR data were resliced to match the spatial orientation of the PET image data, based upon previously published methods [47]. All subjects were scanned on a Siemens ECAT HR+ PET scanner (CTI PET systems, Knoxville, TN) in three-dimensional (3D) imaging mode. The HR+ acquires
Figure 3.5: Typical uncorrected and corrected \([\text{carbonyl}\,^{11}\text{C}]\text{WAY-100635}\) plasma time activity curve

63 continuous slices over a 152-mm axial field of view. Subjects were positioned with the head oriented parallel to the canthomeatal line. A softened thermoplastic mold with generous holes for the eyes, nose, and ears was fitted closely around the head and attached to a headholder to minimize subject motion.

Prior to radiotracer injection, a 5 ml sample of arterial blood was collected and used to assess the level of \([\text{carbonyl}\,^{11}\text{C}]\text{WAY-100635}\) binding to plasma proteins. Immediately following bolus intravenous injection of \(~10\) mCi high-specific activity of \([\text{carbonyl}\,^{11}\text{C}]\text{WAY-100635}\), dynamic emission scanning with arterial blood sampling (input function) was performed over 90 min. The arterial input function was determined from 35 0.5 ml hand-drawn blood samples collected over the scanning interval (1x15 sec., 8x30 sec., 10x1min., 9x5min. and 3x10min.). Blood samples were centrifuged and the plasma radioactivity concentration measured. Additionally, 3 ml 9 blood samples were acquired at after \([\text{carbonyl}\,^{11}\text{C}]\text{WAY-100635}\) injection throughout the study \((0, 1, 2, 5, 10, 30, 45, 60, 90\) min) and used to determine the fraction of unmetabolized \([\text{carbonyl}\,^{11}\text{C}]\text{WAY-100635}\) (of total plasma radioactivity concentration) using high-performance liquid chromatography (HPLC). Plasma data were corrected for the presence of radi-
olabeled metabolites of [carbonyl−11C]WAY-100635 using the HPLC data and input functions were fitted to a sum of exponentials plus a constant term, when necessary. PET data were corrected for radioactive decay and scatter. Image reconstruction was performed using filtered back-projection (Hann filter); the final reconstructed image resolution was 6.5-7.0 mm. A typical reconstructed [carbonyl−11C]WAY-100635 image is given in Fig. 3.6. The scans were visually inspected for head motion and a post processing correction was performed. Head motion was determined by overlaying an MR-based brain outline on each frame of the PET study. The regions of interest (ROI) were hand drawn on the co-registered MR images and applied to the dynamic PET data to generate time activity curves. The following ROIs were selected: Anterior cingulate cortex (ANC), Dorsal Raphe (DRN), Hippocampus (HIP), lateral orbito-frontal cortex (LOF), Mesial temporal Cortex (MTC) and Occipital Cortex (OCC). ROI sampling of the cerebellum was also performed, and this was used as the reference region because of the low concentration of 5-HT1A receptors. The cerebellar reference region (Ref) data were assumed to be representative of the free and nonspecifically bound radioactivity concentrations, in all regions [56].

Figure 3.6: Typical PET image of [carbonyl−11C]WAY-100635 binding of the same middle brain slice (left: time frame number 4; right: time frame number 35).
3. $[^{11}\text{C}]-\text{DASB}$ and $[^{11}\text{C}]\text{carbonyl - }^{11}\text{C}]=\text{WAY-100635}$: Protocol, Subjects and Data
Chapter 4

ROI Modeling

The first part of this chapter is dedicated to all ROI models used to analyse both \([\text{carbonyl}^{11}C]\text{-WAY-100635}\) \([^{11}C]\text{-DASB datasets. Models are divided into Spectral Analysis, models with arterial input function, models without arterial input function and Graphical analysis. In the second part of this chapter results relative to \([^{11}C]\text{-DASB and \([\text{carbonyl}^{11}C]\text{-WAY-100635}\) studies are presented and discussed.}

4.1 Models

4.1.1 I/O Models: the Spectral Analysis

The most widely employed I/O model is the so called Spectral Analysis (SA) Method introduced by Cunningham et al. in 1993 [8] and later generalized by Bertoldo et al. in 1999 [4]. This method is based on the fact that if a system is linear, the impulse response can be written as:

\[
h(t) = \sum_{j=1}^{M} \alpha_j e^{-\beta_j t}
\]  (4.1)
4. ROI Modeling

with \( \beta_j \geq 0 \), for every \( j \), and the tissue tracer concentration \( C_i(t) \) is simply the convolution of \( h(t) \) with the plasma concentration \( C_p(t) \):

\[
C_i(t) = \sum_{j=1}^{M} \alpha_j \int_{0}^{t} C_p(\tau) e^{-\beta_j(t-\tau)} d\tau
\]

The estimation of \( \alpha_j \) and \( \beta_j \) from data provides useful insight into the system behavior. A distinction is made between low, intermediate and high eigenvalues \( \beta_j \) (also referred to as frequency components, thus the term Spectral Analysis). The amplitude \( \alpha \) corresponding to the highest eigenvalue (\( \beta \rightarrow \infty \)) gives a measure of the vasculature within ROI since \( \int_{0}^{t} C_p(\tau) e^{-\beta_j(t-\tau)} d\tau \rightarrow \frac{1}{\beta} C_p(t) \). The number of amplitudes \( \alpha_j \) corresponding to the intermediate \( \beta_j \) gives the number of reversible compartments that can be discriminated in the tissue. However, nothing can be said in terms of compartment connectivity. For example, two amplitudes at the intermediate frequencies do not establish whether the corresponding reversible tissue compartments are parallel (heterogeneous tissue) or in cascade (homogeneous tissue) since these two structures are kinetically indistinguishable. Finally, the amplitude \( \alpha \) corresponding to the lowest eigenvalue (\( \beta \rightarrow 0 \)) reveals the presence of an irreversible process within the region since \( \int_{0}^{t} C_p(\tau) e^{-\beta_j(t-\tau)} d\tau \rightarrow \int_{0}^{t} C_p(\tau) d\tau \).

Thus, the intermediate and low frequency components of the spectrum reflect the extravascular behavior of the tracer or the activity of the tracer within the tissue.

These models, by definition, cannot provide a physiological interpretation of the system but are of tremendous help in the process of model development. In fact, if used in conjunction with sound parameter estimation techniques and parsimony criteria [4], they provide a statistically model independent guide to characterize the reversible and irreversible system components and estimate the minimum number of system compartments.

Components detected with spectral analysis can also be combined to obtain parameters of physiologic interest. For example, Cunningham et al. [8] used the detected components \( \alpha_j \) and \( \beta_j \) to obtain an estimate of the unidirectional clearance of tracer from blood to tissue \( K_1 \), and the volume of
4. ROI Modeling

Figure 4.1: The Three-Tissue Compartment Model

The distribution of the tracer in tissue relative to blood as:

\[ K_1 = h(t = 0) = \sum_{j=1}^{N} \alpha_j \]

\[ V_d = \int_0^\infty h(t)dt = \sum_{j=1}^{N} \frac{\alpha_j}{\beta_j} \]

Sometimes SA is also used to obtain kinetic parameter of the system [62, 63, 41, 10]. However, in this case it is associated with a specific compartmental or noncompartmental system structure and thus gives the same answer of the underlying model.

4.1.2 Compartmental Models with Arterial Input Function

4.1.2.1 The Three-Tissue Compartment Model

Compartmental models with arterial input function use the plasma arterial curve of tracer radioactivity as the known input function. The compartmental model reflecting major kinetic events was first proposed by Mintum et al. in 1984 for the quantitative characterization of regional drug binding sites in vivo [36]. He used baboons injected with fluorine 18-labelled spiperone (\[^{18}F\]spiperone) and multiple PET scans of brain tissue activity to illustrate the quantitative receptor model shown in Fig. 4.1, where \( C_p(t) \) is the arterial
plasma concentration corrected for metabolites, $C_f(t)$ is the tissue concentration of the free ligand, $C_{ns}(t)$ is the tissue concentration of non specifically bound ligand and $C_s(t)$ is the tissue concentration of specifically bound ligand. Parameters $K_1$ [ml ml$^{-1}$min$^{-1}$] and $k_2$ [min$^{-1}$] represent rate constant of ligand transfer from plasma to the tissue and vice versa, while $k_3$ [min$^{-1}$] represents the transfer of tracer to the specifically bound compartment from the free one and $k_4$ [min$^{-1}$] is the return from the specifically bound compartment to the free. $k_5$ [min$^{-1}$] represents the transfer of the tracer from the free to the non specific compartment and $k_6$ [min$^{-1}$] is the return. The model equations are:

$$\frac{dC_f(t)}{dt} = K_1C_p(t) - (k_2 + k_3 + k_4)C_f(t) + k_3C_s(t) + k_6C_{ns}(t)$$
$$\frac{dC_s(t)}{dt} = k_3C_f(t) - k_4C_s(t)$$
$$\frac{dC_{ns}(t)}{dt} = k_5C_f(t) - k_6C_{ns}(t)$$

with initial conditions: $C_f(0) = C_s(0) = C_{ns}(0) = 0$

To better understand the physiological meaning of parameters $k_3$ and $k_4$, let's assume that the binding of the ligand to the receptor site can be described as a bimolecular reaction:

$$k_{on}$$
$$L + R \rightleftharpoons LR$$
$$k_{off}$$

where $L$ represents the ligand, $R$ the receptor site, $LR$ is the binding product, $k_{on}$ is the association rate of the ligand with receptor sites, and $k_{off}$ is the dissociation rate of the specifically bound reaction product. In Fig. 4.1, $C_f(t)$ and $C_s(t)$ represent $L$ and $LR$ respectively. Thus:

$$\frac{dC_s(t)}{dt} = k_{on}C_f(t)C_R(t) - k_{off}C_s(t)$$

where $C_R(t)$ denotes the concentration of receptors. If $B_{max}$ is the total number of available sites, then:

$$B_{max} = C_s(t) + C_R(t)$$
4. ROI Modeling

and, if the ligand is present in tracer concentration, the concentration $C_s$ is negligible. Thus:

$$B_{max} \approx C_r$$

(4.9)

and equation 4.7 becomes:

$$\frac{dC_s(t)}{dt} = k_3 C_f(t) - k_4 C_s(t)$$

(4.10)

with $k_3 = k_{on} B_{max}$ and $k_4 = k_{off}$. An important parameter is also the equilibrium-binding constant $K_D$, which is defined with the ligand-receptor reaction is steady state as:

$$K_D = \frac{C_s}{C_f} = \frac{k_{off}}{k_{on}}$$

(4.11)

The PET scanner measure, $C_i(t)$, is described by:

$$C_i(t) = (1 - V_b)(C_f(t) + C_s(t) + C_{ns}(t)) + V_b C_b(t)$$

(4.12)

where $C_b(t)$ is the whole blood tracer concentration and and $V_b$ [ml ml$^{-1}$] is the vascular volume.

This model is a priori non uniquely identifiable; in particular the identifiability study shows that this model admits two symmetric solutions (see APPENDIX 7). Anyway, if it’s possible to select the correct solution, then, given estimated parameters $K_f$, $k_2$, $k_3$, $k_4$, $k_5$, $k_6$, and $V_b$ the partial and total tracer distribution volumes can be calculated as:

$$V_f = V_1 = \frac{K_1}{k_2 f_1} = \frac{C_f}{C_p}$$

$$V_s = V_2 = \frac{K_1 k_3}{k_2 k_4 f_1} = \frac{C_s}{C_p}$$

$$V_{ns} = V_3 = \frac{K_1 k_5}{k_2 k_6 f_1} = \frac{C_{ns}}{C_p}$$

$$V_t = V_1 + V_2 + V_3 = \frac{K_1}{k_2 f_1} \left( 1 + \frac{k_3}{k_4} + \frac{k_5}{k_6} \right)$$

(4.13)

where $f_1$ is the free plasma concentration at equilibrium and the unit of
4. ROI Modeling

![Diagram of Two-Tissue Compartment Model]

Figure 4.2: The Two-Tissue Compartment Model

distribution volumes is $\text{ml} \cdot \text{ml}^{-1}$.

The binding potential (BP) can be calculated in a selected ROI as $V_s$ normalized to the free distribution volume:

$$BP = \frac{V_s}{V_f} = \frac{k_3}{k_4} = \frac{B_{\text{max}}}{K_D}$$  \hspace{1cm} (4.14)

and it is unitless.

4.1.2.2 The Two-Tissue Compartment Model

When it is possible to assume that the exchange rates between the free tissue and nonspecific binding compartments are sufficiently rapid (compared to the other rates of the model), the Three-Tissue compartment model of Fig. 4.1 (3-TM) can be simplified into the Two-Tissue compartment model shown in Fig.4.2 (2-TM), where

$$C_{f+ns}(t) = C_f(t) + C_{ns}(t)$$  \hspace{1cm} (4.15)

is the free and nonspecific binding tracer concentration.

The model equations are:

$$\frac{dC_{f+ns}(t)}{dt} = K_1 C_p(t) - (k_2 + k_3)C_f(t) + k_4 C_s(t)$$

$$\frac{dC_s(t)}{dt} = k_3 C_{f+ns}(t) - k_4 C_s(t)$$  \hspace{1cm} (4.16)

the initial conditions being: $C_{f+ns}(0) = C_s(0) = 0$. In this case,

$$k_3 = k_{\text{on}} B_{\text{max}} f_2$$  \hspace{1cm} (4.17)
where \( f_2 \) is tracer free fraction in the nondisplaceable compartment.

\[
f_2 \equiv \left( \frac{C_f}{C_f + C_{ns}} \right)_{SS} = \frac{C_f}{C_f + C_{ns}} = \frac{C_f}{C_f(1 + \frac{C_{ns}}{C_f})} = 1 + \frac{k_5}{k_6} \quad (4.18)
\]

PET scanner measure is now described by:

\[
C_i(t) = (1 - V_b)(C_{f+ns}(t) + C_s(t)) + V_bC_b(t) \quad (4.19)
\]

where \( C_b(t) \) is the whole blood tracer concentration and \( V_b \) is the vascular volume. This model is a priori uniquely identifiable and, in addition to parameters \( K_1, k_2, k_3, k_4, \) and \( V_b, \) it is also possible to estimate the partial and total tracer distribution volumes as:

\[
V_{f+ns} = V_1 = \frac{K_1}{k_2f_1} = \frac{C_{f+ns}}{C_p} \\
V_s = V_2 = \frac{K_1k_2}{k_2k_3f_1} = \frac{C_s}{C_p} \\
V_t = V_1 + V_2 = \frac{K_1}{k_2f_1} \left( 1 + \frac{k_3}{k_4} \right) \quad (4.20)
\]

and the binding potential \( BP \) as:

\[
BP = \frac{V_s}{V_{f+ns}} = \frac{f_2B_{max}}{K_D} = \frac{k_3}{k_4} \quad (4.21)
\]

### 4.1.2.3 The One-Tissue Compartment Model

Some tracer cannot be characterized by a compartmental model with three nor two tissue compartments. When this happens, the model that can be used is a further simplification of the Two-Tissue compartment model (Fig. 4.3). The model equation is given by:

\[
\frac{dC_t(t)}{dt} = K_1C_p(t) - k_2C_t(t) \\
C_t(0) = 0 \quad (4.22)
\]

The measured activity is given by:

\[
C_i(t) = (1 - V_b)C_t(t) + V_bC_b(t) \quad (4.23)
\]
4. ROI Modeling

Figure 4.3: The One-Tissue Compartment Model

In this case only parameters $K_1, k_2$ and $V_b$ can be estimated and only the total volume of tracer distribution can be derived as:

$$V_t = \frac{K_1}{k_2} = \frac{C_i}{C_p}$$  \hspace{1cm} (4.24)

This model is referred to 1-TM.

4.1.2.4 Alternative BP Measures

The binding potential calculated as in equations 4.14 and 4.21 should be used only when it is robust and stable, i.e. when it is characterized by a small standard error and a small standard deviation. Otherwise, BP should be calculated from the distribution volumes $V_t$.

If, for example, a region of interest is modeled by a Two-Tissue compartment model and if a region devoid of specific receptors with volume of distribution $V_{tRef} = K'_1/k'_2$ is available, the binding potential can be calculated from:

$$BP = \frac{V_{tROI} - V_{tRef}}{V_{tRef}} = \left[ \frac{K_1}{k_2} \left(1 + \frac{k_3}{k_4}\right) - \frac{K'_1}{k'_2} \right] = \frac{k_3}{k_4}$$  \hspace{1cm} (4.25)

assuming that $K_1/k_2$ ratio is the same for target and reference tissue ($K_1/k_2 = K'_1/k'_2$). This assumption does not imply that $K_1$ and $k_2$ are the same for both regions, only their ratio is. This is plausible, since any higher permeability in one direction should be accompanied with a higher permeability in the other.

The above calculation can also be used if the region of interest can only be fitted to a One-Tissue compartment model. In fact, assuming there is specific
binding (which can be tested by predosing or displacing), the failure of the Two-Tissue compartment model would be due to the specific kinetics. For example, if the exchange between free and bound is fast, it is possible that the model can not detect the specific component as a separate compartment. However, $V_{tROI}$ would still be increased.

Finally, if there is non-specific binding, which is assumed to be the same in the region of interest and in the reference region ($k_5/k_6 = k'_5/k'_6$), the binding potential calculated from distribution volumes will be biased. BP is, in fact, calculated as:

$$BP = \frac{V_{tROI} - V_{tRef}}{V_{tRef}} = \frac{\left[ \frac{k_1}{k_2} \left( 1 + \frac{k_3}{k_4} + \frac{k_5}{k_6} \right) - \frac{k'_1}{k'_2} \left( 1 + \frac{k'_5}{k'_6} \right) \right]}{\frac{k'_1}{k'_2} \left( 1 + \frac{k'_5}{k'_6} \right)}$$

However, the bias is only a scaling factor, provided that the level of non-specific binding is relatively constant [44].

### 4.1.3 Compartmental Models without Arterial Input Function

#### 4.1.3.1 The Reference Tissue Model

The ligand-receptor models described above require the knowledge of the plasma-labelled ligand concentration, which is the forcing function of the models for their identification. A method has been described by Lammertsma et al. [2, 33] (RTM) that allows the quantification of receptor kinetics without measuring the arterial input function. This method relies on the presence of a region without specific binding of ligand that can be considered as reference for all the other regions.

The model is shown in Fig. 4.4, where $C_{Ref}(t)$ is the concentration in the reference tissue and $C_p(t)$ is the plasmatic tracer concentration corrected for metabolites and assumed to be the same for both regions.
Model equations are:

\[
\begin{align*}
\frac{dC_{Ref}(t)}{dt} &= K_1'C_p(t) - k_2'C_{Ref}(t) \\
\frac{dC_{f+ns}(t)}{dt} &= K_1C_p(t) - (k_2 + k_3)C_{f+ns}(t) + k_4C_s(t) \\
\frac{dC_s(t)}{dt} &= k_3C_{f+ns}(t) - k_4C_s(t)
\end{align*}
\]  
(4.27)

with initial conditions:

\[
C_{Ref}(0) = C_{f+ns}(0) = C_s(0) = 0
\]  
(4.28)

and:

\[
\begin{align*}
k_3 &= k_{on}B_{max}f_2 \\
k_4 &= k_{off}
\end{align*}
\]  
(4.29)

This model is a priori uniquely identifiable if \(R_1\) is defined as:

\[
R_1 = \frac{K_1}{K_1'}
\]  
(4.30)

and under the hypothesis that volumes of distributions of non specifically bound tracer in both tissues are the same, meaning:

\[
V_d = \frac{C_{f+ns}}{C_p} = \frac{C_{Ref}}{C_p}
\]  
(4.31)
4. ROI Modeling

Figure 4.5: The Simplified Reference Tissue Model

which brings:

\[
\frac{K_1}{k_2} = \frac{K'_1}{k'_2} \tag{4.32}
\]

The measurement equation is:

\[
C_i(t) = C_{f+ns}(t) + C_s(t) \tag{4.33}
\]

and, under these assumption, the model output is given by:

\[
C_i(t) = C_{f+ns}(t) + C_s(t) = R_1 \left[ C_{Ref}(t) + a \int_0^t C_{Ref}(\tau)e^{-c(t-\tau)}d\tau + b \int_0^t C_{Ref}(\tau)e^{-d(t-\tau)}d\tau \right] \tag{4.34}
\]

where \(C_{Ref}(t)\) is the model input and \(a, b, c, d\) are combinations of parameters \(R_1, k_2, k_3\) and \(k_4\). Four model parameters \(R_1, k_2, k_3\) and \(k_4\) can be estimated and BP can be calculated as:

\[
BP = \frac{k_3}{k_4} \tag{4.35}
\]

4.1.3.2 The Simplified Reference Tissue Model

If the tracer kinetics in the target region are such that it is difficult to distinguish between free and non specifically bound components, the RTM can be simplified as in Fig.4.5 (SRTM). This model equations are:
4. ROI Modeling

\[
\begin{align*}
\frac{dC_{i}(t)}{dt} &= K_1'C_p(t) - k_2^aC_{Ref}(t) \\
\frac{dC(t)}{dt} &= K_1'C_p(t) - k_{2a}C_{i}(t)
\end{align*}
\]  

(4.36)

where: \(C_{i}(t) = C_{f+nS}(t) + C_s(t)\) is the total tracer concentration in the tissue and \(k_{2a}\) is the apparent constant of transfer from specifically bound compartment to plasma. \(k_{2a}\) is related to the parameters \(k_2, k_3, k_4\) of RTM in Fig.4.4 by:

\[
k_{2a} = \frac{k_2}{1 + BP} = \frac{k_2}{1 + \frac{k_3}{k_4}}
\]  

(4.37)

The measurement equation is:

\[
C_{i}(t) = C_{f+nS}(t) + C_s(t)
\]  

(4.38)

ND, assuming that 4.30 and 4.32 still hold, the equation corresponding to 4.34 becomes:

\[
C_{i}(t) = C_{f+nS}(t) + C_s(t) = R_1C_{Ref}(t) + \left[ k_2 - \frac{R_1k_2}{1 + BP} \int_0^t C_{Ref}(\tau)e^{-(k_2/1+BP)(t-\tau)}d\tau \right]
\]  

(4.39)

This model is uniquely identifiable and three parameters \(R_1, k_2\) and \(BP\) can be directly estimated.

4.1.4 The Logan Graphical Analysis

All the ligand-receptor models presented previously require nonlinear identification from the data to quantify the receptor system. Simpler approaches based on graphical methods have been developed, and because of their simplicity they are frequently employed for system quantification. The graphical methods are not dependent upon a particular model structure, but, when a particular compartmental model is assumed for the ligand-receptor system, the graphical methods output can be related to combinations of the compartmental model parameters. A popular graphical method for BP quantification has been proposed by Logan et al. in 1990 [27]. The input required is the
uptake data from a region of interest vs time and an input function that can be either the plasma measurement or the uptake from a suitable reference region.

For reversible systems the form of the graphical analysis model can be derived from the compartmental equations describing tracer accumulation in tissue [27, 5]. When a Two-Tissue compartment model is assumed to describe the kinetics of the system and the plasmatic arterial curve is available (see Fig. 4.2 and equations 4.16), if the PET scanner measures:

\[ C_i(t) = [C_{f+ns}(t) + C_s(t)] + V_b C_b(t) \]  \hspace{1cm} (4.40)

it is possible to rearrange model equation 4.16 into:

\[ \frac{\int_0^t C_i(\tau)d\tau}{C_i(t)} = \frac{m}{C_i(t)} \int_0^t C_p(\tau)d\tau \frac{d\tau}{C_i(t)} + q \]  \hspace{1cm} (4.41)

which has a linear form when the second term \( q \) on the right hand-side of 4.41 is constant. A plot of \( \int_0^t C_i(\tau)d\tau/C_i(t) \) versus \( \int_0^t C_p(\tau)d\tau/C_i(t) \) for times \( t \) is linear after some time \( t^* \) when the exchangeable compartment is in equilibrium with the plasma tracer concentration. The slope of this linear curve is the total tissue distribution volume \( V_t \) plus the plasma contribution:

\[ m = V_t + V_b = \left[ K_1 k_2 \left( 1 + \frac{k_3}{k_4} \right) + V_b \right] = \left[ \frac{K_1}{k_2} (1 + BP) + V_b \right] \]  \hspace{1cm} (4.42)

For a One-tissue compartment model, instead, \( V_t \) is \( K_1/k_2 \), the ratio of transport constants.

When the blood sampling is not available, data from a reference region \( C_{Ref}(t) \) can be used. For the reference region, equation 4.41 becomes:

\[ \frac{\int_0^t C_{Ref}(\tau)d\tau}{C_{Ref}(t)} = V'_t \int_0^t C_p(\tau)d\tau/C_{Ref}(t) + q' \]  \hspace{1cm} (4.43)
4. ROI Modeling

Solving for \( \int_0^t C_p(\tau) d\tau \) and substituting in equation 4.41 one gets:

\[
\frac{\int_0^t C_i(\tau) d\tau}{C_i(t)} = \frac{V_t}{V'_t} \int_0^t C_{Ref}(\tau) d\tau + \frac{V_t C_{Ref}(t) q'}{V'_t} + q
\]  

(4.44)

Again, for times \( t \) after some time \( t^* \) the model in equation 4.44 is linear and it is possible to estimate global parameters:

\[
\alpha_1 = \frac{V_t}{V'_t}
\]
\[
\alpha_2 = \frac{V_t q'}{V'_t}
\]
\[
\alpha_3 = q
\]  

(4.45)

Then, if a particular model structure is assumed, for example 2-TM, BP can be derived from \( \alpha_1 \) as:

\[
BP = \alpha_1 - 1
\]  

(4.46)

Analogous considerations can be made if a 1-TM model structure is considered.

4.2 Parameter Estimation

Except for Graphical analysis, all models require nonlinear identification. In this study all parameters were estimated by weighted nonlinear least squares (WNLLS).

Tissue activity curves are described by:

\[
C_{obs}^i(t_j) = C_i(t_j) + e(t_j)
\]  

(4.47)

where \( j = 1, 2, \ldots, N \), \( t_j \) is the midscan time, \( e(t_j) \) is the measurement error at time \( t_j \), and \( N \) is the number of data.
Thus, the cost function to be minimized is:

\[
WRSS(p) = \sum_{j=1}^{N} w_j \left[ C_{i}^{obs}(t_j) - C_i(p, t_j) \right]
\]  

(4.48)

WRSS denotes weighted residual sum of squares, \( w_j \) is the weight of the datum, and \( p \) the vector of unknown model parameters of dimension \( P \). Measurement error was assumed to be additive, uncorrelated, Gaussian, zero mean, and with a variance described as proposed in [35]:

\[
\sigma^2(t_j) = \gamma \frac{C_{i}^{obs}(t_j)}{\Delta t_j}
\]  

(4.49)

where \( \Delta t_j \) is the length of the scanning interval relative to \( C_{i}^{obs}(t_j) \) and \( \gamma \) is an unknown proportionality constant.

Weights were chosen optimally [7] as:

\[
w_j = \frac{\Delta t_j}{C_{i}^{obs}(t_j)}
\]  

(4.50)

and the scale factor \( \gamma \) of equation 4.49 was estimated a posteriori [7] as:

\[
\gamma = \frac{WRSS(\hat{p})}{N - P}
\]  

(4.51)

where \( WRSS(\hat{p}) \) is the value of the cost function evaluated at the minimum, i.e., for \( p \) equal to the vector of estimated model parameters \( \hat{p} \):

\[
WRSS(\hat{p}) = \sum_{j=1}^{N} w_j \left[ C_{i}^{obs}(t_j) - C_i(\hat{p}, t_j) \right]
\]  

(4.52)

WRSS has been minimized by using the Levenberg- algorithm as implemented in [1].

Precision of the parameter estimates was evaluated from the inverse of
4. ROI Modeling

the Fisher information matrix $M$ by:

$$COV(\hat{p}) = \gamma M^{-1}$$ (4.53)

4.3 $[^{11}\text{C}]-$DASB Modeling Results

4.3.1 Spectral Analysis

Spectral Analysis was performed at ROI level on 6 healthy elderly controls. Based on literature information [48, 70], the ligand-receptor system was described with a one- and a two-exponential models (respectively $SA_1$, $SA_2$). The mean AIC (Akaike Information Criterion) values for $SA_1$ and $SA_2$ were calculated as implemented in SAAM II (a simulation, analysis and modeling software for kinetic analysis developed at the University of Washington [20]) and are reported in Table 4.1.

<table>
<thead>
<tr>
<th>Method</th>
<th>AIC $SA_1$</th>
<th>AIC $SA_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean ± SD</td>
<td>mean ± SD</td>
</tr>
<tr>
<td>AMY</td>
<td>-2.19 ± 0.27</td>
<td>no fit</td>
</tr>
<tr>
<td>CER</td>
<td>-2.13 ± 0.34</td>
<td>no fit</td>
</tr>
<tr>
<td>DRN</td>
<td>-1.83 ± 0.26</td>
<td>no fit</td>
</tr>
<tr>
<td>HIP</td>
<td>-2.29 ± 0.36</td>
<td>no fit</td>
</tr>
<tr>
<td>MTC</td>
<td>-2.32 ± 0.35</td>
<td>no fit</td>
</tr>
<tr>
<td>THL</td>
<td>-1.98 ± 0.45</td>
<td>no fit</td>
</tr>
</tbody>
</table>

Table 4.1: Spectral Analysis Results. AIC values.

As shown in table 4.1, $SA_2$ never converged for all subjects in all regions, and since only the one-exponential model reached convergence, a single reversible tissue compartment in the ROI compartmental model structure was expected.
4. ROI Modeling

4.3.2 Compartmental Models with Arterial Input Function

Based on SA results, regional brain uptake curves of $[^{11}C]$-DASB were quantified using either a One-Tissue compartment model or a constrained Two-tissue compartment model. Six different kinetic compartmental models with arterial input function, involving two to four rate constants, were identified with weighted non linear least squares fitting (WNLLS) analysis using SAAM II [20] and Matlab (The Mathworks, [21]). Cerebral Blood volume was either considered an unknown parameter to be estimated or was either fixed to 5% for all ROIs [31].

The different model configurations are indicated in Table 4.2. Method A is the standard 1-TM, and WNLLS analyses were performed to estimate $K_1$, $k_2$, $V_b$ and the total volume of distribution as in section 4.1.2.3.

<table>
<thead>
<tr>
<th>Method</th>
<th>Number of Tissue compartments</th>
<th>Constraints</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>None</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>$V_b = 5%$</td>
</tr>
<tr>
<td>C</td>
<td>2</td>
<td>$k_4 = 0$</td>
</tr>
<tr>
<td>D</td>
<td>2</td>
<td>$k_4 = 0$ $V_b = 5%$</td>
</tr>
<tr>
<td>E</td>
<td>2</td>
<td>$V_t(E) = V_t(B)$ $V_b = 5%$</td>
</tr>
<tr>
<td>F</td>
<td>2</td>
<td>$V_{f+ns}(F) = V_{tRef}$ $V_b = 5%$</td>
</tr>
</tbody>
</table>

Table 4.2: Fitting strategies used for kinetic modeling of $[^{11}C]$-DASB

Method B is still based on the 1-TM model but in this case the blood volume $V_b$ was fixed to 5%. Methods C, D, E and F are all based on a 2-TM model with rate constants $k_4$ set equal to zero for methods C and D. For method C, WNLLS analyses were performed to estimate the four parameters $K_1$, $k_2$, $k_3$ and $V_b$ and to derive the binding potential as in equation 4.25. In method D the blood volume was also set equal to 5%, and more constraints were considered in methods E and F. In method E besides the blood volume constraint, the total volume of tracer distribution of the 2-TM model was set equal to the total volume of tracer distribution of the 1-TM model, while in method F the distribution volume of the nondisplaceable compartment in

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4. ROI Modeling

the 2-TM model was set equal to the total volume distribution of a reference region (i.e. the Cerebellum), which is assumed to be described by a 1-TM model. When possible the binding potential BP was calculated as in equations 4.25. Goodness of fit was evaluated and Akaike Information Criterion (AIC) values were compared.

The parameter estimation process was difficult for methods C, D, E and F. These methods failed to converge in most cases or produced very poor parameter precisions while the estimation process gave acceptable results for methods A and B. Therefore, only results for these two latter methods will be reported and discussed. Table 4.3 shows mean regional estimated parameter values for methods A and B.

For method A, tissue time activity curves were well-described by a 1-TM model and convergence was achieved in all subjects for all regions. Rate constant $K_1$ ranged from a maximum of 0.73 ($mlml^{-1}min^{-1}$) in the Thalamus (THL) to a minimum of 0.40 in the Amygdala (AMY). $K_1$ values were identified precisely in all regions with CV values always less than 4%. Rate constant $k_2$ showed to be very consistent between regions (between 0.01 and 0.02 ($min^{-1}$)) except for Cerebellum (CER) where it was higher (mean value 0.04 calculated over the six subjects). This parameter was also identified with good precision in all regions with CV values always between 2% in CER, HIP (hippocampus) and THL and 9% in DRN (the dorsal Raphe region). The rank order for total volumes of distributions for method A was: DRN>THL>AMY>MTC>HIP>CER, in perfect agreement with the known distribution of the serotonin transporter. The blood volume $V_b$ was also estimated in all regions but with greater uncertainty. It ranged from 0.02 in AMY to 0.06 in CER and THL with CVs varying form 54% for THL to 375% in AMY.

In method B, $V_b$ was fixed to 5%. Rate constant $K_1$ also ranged from a maximum of 0.71 ($mlml^{-1}min^{-1}$) in the THL to a minimum of 0.41 in AMY. $K_1$ values were identified with good precision in all regions with CV values always less than 2%. Rate constant $k_2$ was, again, consistent between regions (between 0.01 and 0.02 ($min^{-1}$)) except for Cerebellum (CER) where it was higher (mean value 0.04). $k_2$ was well identified in all regions with CV values
<table>
<thead>
<tr>
<th>Method</th>
<th>$V_b$ (ml/ml)</th>
<th>CV (ml/ml)</th>
<th>$K_1$ (ml/ml/min)</th>
<th>CV (min^-1)</th>
<th>$k_2$ (min^-1)</th>
<th>CV</th>
<th>$V_t$ (ml/ml)</th>
<th>CV</th>
<th>AIC</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMY</td>
<td>0.02</td>
<td>(375)</td>
<td>0.40</td>
<td>(3)</td>
<td>0.01</td>
<td>(4)</td>
<td>30.00</td>
<td>(4)</td>
<td>-2.18</td>
<td>0.27</td>
</tr>
<tr>
<td>CER</td>
<td>0.06</td>
<td>(61)</td>
<td>0.61</td>
<td>(4)</td>
<td>0.04</td>
<td>(2)</td>
<td>15.73</td>
<td>(3)</td>
<td>-1.72</td>
<td>1.19</td>
</tr>
<tr>
<td>DRN</td>
<td>0.04</td>
<td>(100)</td>
<td>0.47</td>
<td>(4)</td>
<td>0.01</td>
<td>(9)</td>
<td>70.57</td>
<td>(9)</td>
<td>-1.91</td>
<td>0.25</td>
</tr>
<tr>
<td>HIP</td>
<td>0.04</td>
<td>(89)</td>
<td>0.48</td>
<td>(3)</td>
<td>0.02</td>
<td>(2)</td>
<td>21.52</td>
<td>(4)</td>
<td>-2.41</td>
<td>0.38</td>
</tr>
<tr>
<td>MTC</td>
<td>0.04</td>
<td>(99)</td>
<td>0.47</td>
<td>(3)</td>
<td>0.02</td>
<td>(3)</td>
<td>24.12</td>
<td>(4)</td>
<td>-2.43</td>
<td>0.37</td>
</tr>
<tr>
<td>THL</td>
<td>0.06</td>
<td>(54)</td>
<td>0.73</td>
<td>(4)</td>
<td>0.02</td>
<td>(2)</td>
<td>34.70</td>
<td>(4)</td>
<td>-2.12</td>
<td>0.37</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Method</th>
<th>$V_b$ (ml/ml)</th>
<th>CV (ml/ml)</th>
<th>$K_1$ (ml/ml/min)</th>
<th>CV (min^-1)</th>
<th>$k_2$ (min^-1)</th>
<th>CV</th>
<th>$V_t$ (ml/ml)</th>
<th>CV</th>
<th>AIC</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMY</td>
<td>0.05 Fixed</td>
<td>0.41</td>
<td>(2)</td>
<td>0.01</td>
<td>(4)</td>
<td></td>
<td>30.98</td>
<td>(3)</td>
<td>-2.20</td>
<td>0.28</td>
</tr>
<tr>
<td>CER</td>
<td>0.05 Fixed</td>
<td>0.60</td>
<td>(2)</td>
<td>0.04</td>
<td>(2)</td>
<td></td>
<td>15.67</td>
<td>(1)</td>
<td>-2.25</td>
<td>0.34</td>
</tr>
<tr>
<td>DRN</td>
<td>0.05 Fixed</td>
<td>0.47</td>
<td>(2)</td>
<td>0.01</td>
<td>(9)</td>
<td></td>
<td>71.45</td>
<td>(7)</td>
<td>-1.92</td>
<td>0.26</td>
</tr>
<tr>
<td>HIP</td>
<td>0.05 Fixed</td>
<td>0.48</td>
<td>(1)</td>
<td>0.02</td>
<td>(2)</td>
<td></td>
<td>21.63</td>
<td>(1)</td>
<td>-2.77</td>
<td>0.07</td>
</tr>
<tr>
<td>MTC</td>
<td>0.05 Fixed</td>
<td>0.47</td>
<td>(1)</td>
<td>0.02</td>
<td>(2)</td>
<td></td>
<td>24.38</td>
<td>(1)</td>
<td>-2.77</td>
<td>0.21</td>
</tr>
<tr>
<td>THL</td>
<td>0.05 Fixed</td>
<td>0.71</td>
<td>(1)</td>
<td>0.02</td>
<td>(2)</td>
<td></td>
<td>34.03</td>
<td>(1)</td>
<td>-2.27</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Table 4.3: regional results for methods A and B (mean values and coefficient of variations)
always between 2% in CER, HIP, MTC and THL and 9% in DRN. The rank order for total volumes of distributions is the same as method A.

For what concerns the binding potential, BP was calculated, for both methods A and B, as in equation 4.25. Fig. 4.6 represents BP (mean±SD) regional estimates for methods A and B. As can be seen in Fig. 4.6, methods A and B gave identical BP values regardless of $V_b$. Moreover, as expected, BP was high in high receptor density regions as DRN (3.43±0.75 for method A and 3.53±0.70 for method B) and low in low density regions as HIP (0.38±0.13 for method A and 0.40±0.19 for method B).

Based on parameter precision and the AIC values, the arterial model selected for $[^{11}\text{C}]-\text{DASB}$ kinetics in all ROIs in elderly healthy controls is the One-tissue compartment with $V_b$ set equal to 5% (i.e. method B).

### 4.3.3 Compartmen tal Models without Arterial Input Function

Compartmental models without arterial input function were also evaluated. RTM always converged except in HIP, DRN and THL where it converged in 5 of the 6 subjects. SRTM, instead, converged in all regions for all subjects.

Results (mean values and precision) for the standard reference tissue model (RTM) and its simplified version (SRTM) are shown in Table 4.4.
## 4. ROI Modeling

<table>
<thead>
<tr>
<th>Method</th>
<th>R1</th>
<th>CV</th>
<th>k2</th>
<th>CV</th>
<th>k3</th>
<th>CV</th>
<th>BP</th>
<th>CV</th>
<th>AIC</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMY</td>
<td>0.74 (5)</td>
<td>0.06 (69)</td>
<td>0.04 (168)</td>
<td>0.06 (170)</td>
<td>0.78 (7)</td>
<td>-2.59</td>
<td>0.18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DRN</td>
<td>0.73 (8)</td>
<td>0.11 (49)</td>
<td>0.04 (80)</td>
<td>0.02 (133)</td>
<td>4.84 (54)</td>
<td>-2.02</td>
<td>0.22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIP</td>
<td>0.80 (3)</td>
<td>0.06 (54)</td>
<td>0.02 (158)</td>
<td>0.07 (159)</td>
<td>0.36 (91)</td>
<td>-2.95</td>
<td>0.06</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTC</td>
<td>0.78 (2)</td>
<td>0.06 (37)</td>
<td>0.03 (98)</td>
<td>0.06 (103)</td>
<td>0.52 (9)</td>
<td>-3.06</td>
<td>0.21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>THL</td>
<td>1.21 (5)</td>
<td>0.18 (42)</td>
<td>0.08 (32)</td>
<td>0.09 (32)</td>
<td>1.05 (2)</td>
<td>-2.51</td>
<td>0.14</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Method</th>
<th>R1</th>
<th>CV</th>
<th>k2</th>
<th>CV</th>
<th>BP</th>
<th>CV</th>
<th>AIC</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMY</td>
<td>0.73 (2)</td>
<td>0.04 (7)</td>
<td>0.79 (6)</td>
<td>-2.59</td>
<td>0.19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DRN</td>
<td>0.75 (3)</td>
<td>0.03 (8)</td>
<td>2.37 (14)</td>
<td>-2.02</td>
<td>0.23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIP</td>
<td>0.79 (1)</td>
<td>0.03 (9)</td>
<td>0.38 (8)</td>
<td>-2.91</td>
<td>0.09</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTC</td>
<td>0.63 (3)</td>
<td>0.03 (6)</td>
<td>0.48 (5)</td>
<td>-3.01</td>
<td>0.22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>THL</td>
<td>1.14 (2)</td>
<td>0.05 (10)</td>
<td>1.03 (3)</td>
<td>-2.29</td>
<td>0.22</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4.4: Compartmental models without Arterial Input Function Results
Indeed, the RTM method, when it converges, gives precise estimates of parameter $R_1$, $k_2$, and $BP$, while the other rate constants ($k_3$ and $k_4$) are very poorly estimated. $R_1$ ranges from a minimum of 0.73 in AMY to a maximum of 1.21 in THL and the parameter precision in always between 2% and 8%. $k_2$ ranges from a minimum of 0.06 in AMY, HIP and MTC to a maximum of 1.18 in THL and its precision in always between 37% and 69%. $BP$ is very low in HIP and MTC (0.36 and 0.52 respectively) while becomes very high in DRN (4.84). $BP$ precision, although still acceptable, is very variable ranging from 2% in THL to 91% in HIP. $k_3$ and $k_4$ are very unstable parameters. $k_3$ is associated to mean CVs higher than 100% in regions HIP and AMY, while in DRN and MTC CVs are 80% and 98% respectively. $k_4$ is even more unstable since the only region associated to a CV value less than 100% was THL.

The estimates are more precise with SRTM. In this case, though, only $R_1$, $k_2$ and $BP$ can be obtained. $R_1$ is very well estimated and ranges from a minimum of 0.73 (SD is 0.08) in AMY to a maximum of 1.14 (SD is 0.14) in THL and its parameter precision in always between 1% and 3%. $k_2$ now is more stable ranging from a minimum of 0.03 to a maximum of 0.05 in THL and its precision in always between 6% and 10%. $BP$ is very low in HIP ($0.38 \pm 0.13$, mean±SD) and MTC ($0.48 \pm 0.12$, mean±SD) while becomes very high in DRN ($2.38 \pm 1.26$, mean±SD), even if less than what found by RTM. $BP$ precision, now is very high with CV ranging from 3% in THL to 14% in DRN. The rank order for BP evaluated by SRTM was: DRN$\gg$THL$\gg$AMY$>$MTC$>$HIP, again in perfect agreement with the known distribution of the serotonin transporter.

Finally, AIC values are very similar between the two methods both in values and SD. Anyway, the more stability of SRTM with respect to RTM makes it the preferred between the reference tissue models.

4.3.4 Logan Graphical Analysis

The Logan graphical analysis was performed in all subjects. For this method it is only possible to obtain an estimate of the total volume of tracer distribution $V_t$ and the binding potential $BP$ as $(k_3/k_4)$ derived by equation
4. ROI Modeling

Table 4.5: The Logan Graphical Method: Results

<table>
<thead>
<tr>
<th>Method</th>
<th>$V_t$ (min$^{-1}$)</th>
<th>BP (unitless)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Logan</td>
<td>mean ± SD</td>
<td>mean ± SD</td>
</tr>
<tr>
<td>AMY</td>
<td>27.57 ± 2.48</td>
<td>0.90 ± 0.43</td>
</tr>
<tr>
<td>CER</td>
<td>15.44 ± 1.88</td>
<td>-</td>
</tr>
<tr>
<td>DRN</td>
<td>70.70 ± 27.47</td>
<td>3.49 ± 1.35</td>
</tr>
<tr>
<td>HIP</td>
<td>21.28 ± 1.60</td>
<td>0.39 ± 0.17</td>
</tr>
<tr>
<td>MTC</td>
<td>23.53 ± 1.75</td>
<td>0.54 ± 0.23</td>
</tr>
<tr>
<td>THL</td>
<td>32.12 ± 6.73</td>
<td>1.08 ± 0.37</td>
</tr>
</tbody>
</table>

4.46. The blood volume term $V_b$ was considered equal to 5% in this case too. Results are shown in Table 4.5. Again, the rank order for total volumes of distributions is: DRN > THL > AMY > MTC > HIP > CER. There is also a good correspondence between $V_t$ and $BP$ values calculated with this method and arterial methods A and B, while the Logan methods gives intermediate estimates between RTM and SRTM.

4.3.5 Discussion

Spectral analysis suggested the presence of a single tissue compartment in all ROIs.

Six, compartmental model with arterial input function were put in competition. Of the six only two (A and B) based on 1-TM gave acceptable results. In particular, method B performed better than methods A in terms of model fits, pattern of weighted residuals, precision of estimated parameters and AIC values. For both models the transport parameter $K_1$ was estimated precisely in all regions. The total distribution volume $V_t$ was also calculated with high precision and, although this parameter includes information both on radioligand delivery and binding to specific receptors, at equilibrium it is independent of tracer delivery and gives an index of receptor density. This assumption is supported by the observation that the rank order of $V_t$ values very well correlated with SERT densities rank found in the human brain postmortem [40]. For both methods $V_t$ in CER was high as compared with that of other neuroreceptor radioligands. This is in agreement with Ginovart.
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et al. [48] and is a common characteristic of several SERT radioligands.

Two compartmental models without arterial input function were also evaluated. SRTM, although providing less parameters, performed better than the RTM, for which parameters $k_3$ and $k_4$ were very poorly identified with CV in most cases greater than 100%. These reference tissue models give different estimates from arterial methods (i.e. B) in terms of $k_2$ and $BP$. In particular, with respect to model B, RTM heavily overestimates $k_2$ in all regions while a $BP$ overestimation is evident only in DRN ($\sim37\%$). In all the remaining regions RTM is underestimating. In particular the underestimation is $\sim30\%$ in AMY and within 10% in the other ROI. SRTM still overestimates $k_2$ in all regions but less than RTM and with a modest $BP$ underestimation in all regions except DRN ($\sim30\%$). Certainly DRN is a very small and noisy region, therefore this variability in $BP$ estimation is not surprising.

Logan graphical analysis results are in agreement with that of method B. In particular $V_t$ is only slightly underestimated by Logan method, with an underestimation between 1% (in DRN) and 11% in AMY. With respect to methods RTM and SRTM, Logan graphical method introduces less bias.

Results reported in this study are not in disagreement with those reported in [48] and [70]. One important difference is that in both these studies young healthy subjects were studied, while here elderly healthy subjects were investigated. Frankle and collaborators analyzed different methods for the serotonin transporter quantification from $[^{11}C]-DASB[68]$. Method 1-TM and 2-TM yielded very similar results in all subjects and regions and 2-TM vs 1-TM was recommended based on the observation that 2-TM fits better for some dataset and there is no apparent loss of stability with 2-TM compared with 1-TM. One possible explanation of this discrepancy could be the PET scanning time, which was 120 min in Frankle study, while it was 90 min in this study. Of note is that, due to the physiological decay of $^{11}C$, as well as subject head motion, the data becomes increasingly noisy towards the end of the scan. Frankle et al. [68], anyway, unlike Ginovart et al. [48], did not find reduction in values of $V_t$ derived with the standard Logan graphical analysis when compared with kinetic analysis. This is in agreement with what found in this study, where Logan $V_t$ underestimation was always between -11% and
Table 4.6: AIC values from the I/O modeling. In HIP $SA_3$ convergence is achieved only in 2 of the 9 subjects, while in DRN in only 4 of the 9 subjects

-1\% in AMY and CER, DRN and HIP regions respectively. For what concerns SRTM method, Frankle et al. [68] also found $BP$ values in most regions similar to those achieved with 1-TM. This is, again, in agreement with what found in this study except for region DRN, where BP underestimation is marked with respect to model 1-TM (method B).

In summary, with arterial function available, the best model for $[^{11}\text{C}]$-DASB kinetics quantification in elderly healthy subjects is method B (1-TM with $V_b$ set equal to 5\%), even though graphical analysis only introduces minimal bias. If arterial curve is not available, then, the method of choice for $[^{11}\text{C}]$-DASB kinetics quantification in elderly healthy subjects is SRTM, which introduces less bias than RTM.

### 4.4 $[^{11}\text{C}]$WAY-100635 Modeling Results

#### 4.4.1 Spectral Analysis

ROI Spectral Analysis was applied on the 9 young healthy controls. The ligand-receptor system was described with a one-, a two- and a three-exponential model (respectively $SA_1$, $SA_2$ and $SA_3$). The mean AIC (Akaike Information Criterion) values were calculated as implemented in SAAM II and are reported in Table 4.6. Based on the AIC values, the three-exponential model was selected as the most parsimonious for describing all ROI activities except
4. ROI Modeling

Figure 4.7: Different UF curves for a representative subject. Dashed line is the exponentially interpolated UF ($UF_{Exp}$). Solid line is the linearly interpolated UF ($UF_{Lin}$). Inner graph is a $0 - 5$ min zoom.

for DRN and HIP, where a two exponential model was selected. This result suggests the presence of three reversible tissue compartments in most ROIs of $[{\textit{carbonyl}}-{^{11}}\text{C}]$WAY-100635.

One factor that could affect SA is the model used for the unchanged tracer fraction (UF) during the metabolite correction process. Although many models can be used to describe UF, usually, $[{\textit{carbonyl}}-{^{11}}\text{C}]$WAY-100635 UF is modeled in two different ways: with a linear or an exponential model. In order to understand if and how much different unmetabolized plasma function modeling affects the quantification of $[{\textit{carbonyl}}-{^{11}}\text{C}]$WAY-100635 images, UF data of four of the nine subjects were modeled both with a linear interpolation ($Lin$) and exponential model ($Exp$) and metabolite-corrected plasma activity curves ($C_{pLin}$ and $C_{pExp}$ respectively) were calculated. SA, then, was performed with metabolite-corrected plasma activity curves $C_{pLin}$ and $C_{pExp}$ as input function on ROIs ANC, CER, DRN, HIP, LOF, MTC and OCC. Results indicate that different UF modeling produces different metabolite-corrected plasma activity curves. For instance, Fig. 4.7 shows, for a representative subject, the two different curves obtained modeling UF data by using a linear interpolation or an exponential model. The two different interpolated UF curves resolve in two different metabolite-corrected arterial input functions (Fig. 4.8), and an important difference between the two different metabolite-corrected arterial plasma curves was noted in all four subjects.
4. ROI Modeling

Figure 4.8: Different shapes of the metabolite-corrected arterial input function curve for a representative. Dashed line is the corrected arterial function calculated by $UF_{Exp}$ (i.e. $C_{pExp}$). Solid line is the corrected arterial function calculated by $UF_{Lin}$ (i.e. $C_{pLin}$). Inner graph is a $0 - 10$ min zoom.

during the first 5 minutes of the experiment while it was undetectable afterward. By comparing the eigenvalues output of the SA, one could conclude that when the difference (evaluated as 0-5 min area under the curve, $AUC$, difference in percent) between $C_{pLin}$ and $C_{pExp}$ was less than or equal to 30% (in 3 of the 4 subjects), SA returned the same three components (same amplitudes at the same frequencies) in the same intermediate frequency range. Fig. 4.9 shows the spectral lines obtained by using $C_{pLin}$ and $C_{pExp}$ together with their numerical estimated values (i.e. amplitudes $A_i$ and eigenvalues $a_i$) for that subject. Otherwise, When the difference between $AUC$ $C_{pLin}$ and $C_{pExp}$ was $> 30\%$ (in only one of the four subjects), SA returned two different spectra. In particular: when $C_{pLin}$ was considered, then SA indicated the presence of 3 distinct components in the intermediate frequencies in all ROIs; when $C_{pExp}$ was used, then SA still showed the presence of 3 distinct components but in different intermediate frequencies for regions ANC, CER, LOF and OCC region, and only 2 distinct components of intermediate frequencies were returned for regions HIP and MTC (see Fig. 4.10). Results indicate, then, that the model used to obtain 1-carbonyl $\text{[C]WAY-100635}$ metabolite-corrected plasma activity curves has potentially an impact on the quantification of the spectrum components if the difference between 0–5 min $AUC$ $C_{pLin}$ and $C_{pExp}$ is greater than 30%. In particular, the use of $C_{pLin}$ or
4. ROI Modeling

Figure 4.9: SA results when the 0–5 min AUC difference is less than 30% (regions ANC and MTC).

Figure 4.10: SA results when the 0–5 min AUC difference is greater than 30% (regions ANC and MTC).
4. ROI Modeling

\( C_{pExp} \) provides a different spectrum in terms of the number of components. Fortunately, when the 5 min \( AUC \) difference between \( C_{pLin} \) and \( C_{pExp} \) is less that 30\%, SA gives the same results. The employment of a Hill type function proposed by Gunn et al. [57] to fit UF data was also considered. Results are not shown for sake of space, but the analysis indicated that the Hill modeling results were intermediate between the linear and exponential ones.

Since UF modeling proved to potentially affect the selection of the compartmental model to quantify \([ carbonyl -^{11} C]\)WAY-100635 images, SA was rerun for all nine subjects using both the linear and exponential UF interpolation. Fortunately, mean results confirmed the three-exponential model as the most parsimonious in all ROIs except for HIP and DRN regions regardless of the UF model (see Fig. 4.11 and 4.12).

4.4.2 Compartmental Models with Arterial Input Function

Regional brain uptake curves of \([ carbonyl -^{11} C]\)WAY-100635 were quantified using three different kinetic arterial compartmental models based on either Two- or Three-tissue compartments and involving five or six rate constants. UF modeling, as explained in the previous section, proved not to affect the analysis and the arterial input function considered here is corrected for metabolites modeled as a sum of exponentials. Weighted non linear least squares fitting (WNLLS) analysis using SAAM II and Matlab was performed. Cerebral blood volume was either considered an unknown parameter to be estimated or was fixed to 5\% for all ROIs [31]. Bayesian constraints on rate constants \( k_5 \) and \( k_6 \) were also considered.

The different model configurations are indicated in Table 4.7.
4. ROI Modeling

Figure 4.11: SA results in low to intermediate receptor density regions (CER and LOF)
Figure 4.12: SA results in high receptor density regions (HIP and DRN)
4. ROI Modeling

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<tr>
<td>B</td>
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<tr>
<td>C</td>
<td>3</td>
<td>$V_b = 5% + \text{bayesians on } k_5 \text{ and } k_6$</td>
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Table 4.7: Fitting strategies used for kinetic modeling of $[^{11}\text{C}]$WAY-100635

Method A is the standard 2-TM and WNLLS analysis was performed to estimate parameters $K_1$, $k_2$, $k_3$, $k_4$ and $V_b$. The volume of distribution in each compartment and the total volume of tracer distribution were derived and the binding potential was calculated as the normalized difference between distribution volumes in the target and reference region. Method B is the standard 3-TM model. WNLLS analysis was performed to estimate parameters $K_1$, $k_2$, $k_3$, $k_4$, $k_5$, $k_6$ and $V_b$. As stated in section 4.1.2.1 this model admits two symmetric solutions and, by comparing the plots of single compartmental kinetics, it was possible to understand which of the two symmetric solutions of the model was the correct one. Once each rate constant was given the correct value, it was possible to derive the volume of distribution in each compartment and the total distribution volume. The binding potential was then derived as the ratio $k_3/k_4$, and also was calculated as for methods A. Finally, method C is a constrained version of 3-TM model, where the blood volume was set equal to 5% and were Bayesian constraints were applied to rate constants $k_5$ and $k_6$. In particular, the Bayesian distribution for $k_5$ was associated to a mean value given by the mean of all $k_5$ estimated with method A and a standard deviation given by the standard deviation of all $k_5$ from method B. The same for rate constant $k_6$. For method C WNLLS estimation allowed the quantification of parameters $K_1$, $k_2$, $k_3$, $k_4$, $k_5$, $k_6$ and $V_b$. Then the single compartment and total distribution volumes were derived and the binding potential was calculated as for method B.

The parameter estimation process was successful for all methods in almost all regions. This was expected since the SA had selected as the most parsimonious model for $[^{11}\text{C}]$WAY-100635 kinetics, in all regions except HIP and DRN, a 3-TM model. Results relative to the standard 2-TM
4. ROI Modeling

models (method A) are reported in Table 4.8. As can be seen in Table 4.8 all parameter were well estimated with high precision. Results relative to method B are reported in Table 4.9. In HIP, though, method B converged only in 5 of the 8 subjects and in DRN it converged only in 2 of the 8 subjects with very high CVs.

In region CER method B converged in 5 of the 9 subjects with good CVs; in region ANC method B converged in 5 of the 9 subjects, in region MTC and OCC it converged in 6 of the 9 subjects and in 7 of the 8 subjects respectively, while in region ANC and LOF method B converged in all subjects with good CVs. As can be seen in Table 4.9, all parameters are well estimated with high precision and distribution volumes are in agreement with the compartmental interpretation of the standard Three-tissue compartment model (see section 4.1.2.1). Note, also, that in HIP, whenever method B converged, it gave very good estimates of all parameters with small CVs and small variability (for example, $V_b$ was 0.06±0.02, $k_3$ was 0.28±0.08, $k_6$ 0.19±0.13 and $BP_{ratio}$ was 12.71±4.22 (mean±SD values) ). Results relative to method C are also reported (see Table 4.10). It has to be noted that the Bayesian constraints helped in improving model convergence. With method C convergence was achieved for all subjects in all regions except DRN where only 1 of the 8 subjects couldn't converge.

Mean AIC values (calculated on subjects for all regions) and standard deviations (between subjects) for methods A and B and C are reported in Table 4.11. Lower AIC values refer to more parsimonious models and Table 4.11 shows that method B is characterized by regional mean AIC values that are always lower than method A (except for DRN). Method C, also, has higher AIC values than method B in all regions except ANC, where the mean AIC value is equal to that of method B but has a greater between subjects variability (0.44 against 0.18 in method B). From Table 4.11 it is possible to notice that method C does not improve the fit in terms of AIC respect to method B, even though now the DRN and HIP regions can be identified. Therefore, by considering the precisions of the estimated parameters, the AIC values, model fits and weighted residual plots, it is possible to conclude that, method B is the one that best describes tracer kinetics of
Table 4.8: The Two-Tissue Compartment Model: Results. BPratio is the binding potential calculated as the normalized difference between the target and reference region. \( V_1 \) is the distribution volume in the nondisplaceable compartment and \( V_2 \) is the distribution volume in the specific compartment.

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Metho d V

4. ROI Modeling
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Table 4.9: The Three-Tissue Compartment Model (method B): Results
Table 4.10: The Constrained Three-Tissue Compartment Model: Results

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4. ROI Modeling

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Table 4.11: Mean AIC values for methods A, B and C

Figure 4.13: Estimated BP values with methods A and B

[carbonyl$^{-11}$C]WAY-100635 in all regions (CER included) except DRN and HIP where method A is the best. The different kinetic behavior of these latter regions could have a physiological explanation. In fact, being them regions characterized by a high radioligand uptake, in these regions the specific binding component could be predominant while the free and non specific components, being much lower, cannot be easily kinetically separated from the specific one.

BP estimates ($k_3/k_4$) with methods A and B are shown in Fig. 4.13. Relative to method B, BP values calculated with 2-TM (method A) are lower especially in high receptor density regions (~37% in ANC, ~25% in
4. ROI Modeling

Figure 4.14: Predicted dynamics in region ANC for two representative subjects (#2 and #4)

LOF, ~15% in OCC, ~43% in HIP and ~50% in MTC). CER has a different behavior with respect to all the other ROIs since it is generally assumed that CER is a region devoid of specific $5-HT_{1A}$ receptors. CER will be examined below.

The analysis also evidenced that the standard 2-TM model is characterized, for [carbonyl $^{11}$C]WAY-100635, by compartmental kinetics that do not follow the standard physiological interpretation. In fact, if the standard Three-Tissue compartment model is considered where compartment 1 is the free tracer in the tissue, compartment 2 is the non specific binding of the tracer and compartment 3 is the specific binding, and if the Two-Tissue compartment model is also considered, if kinetics from compartment 1 of 3-TM and compartment 1 of 2-TM are compared (see Fig. 4.14 and 4.15: Two-tissue model (upper graph) and Three-tissue model (lower graph)), it becomes evident that these kinetics are almost identical. Moreover, compartment 2 of model 2-TM is almost the sum of kinetics in compartments 2 and 3 of model 3-TM and, therefore, represents a combination of specific and part of nonspecific binding. Analogous considerations can be done for all nine subjects.

Particular attention requires the discussion of results about Cerebellum.
4. ROI Modeling

CER was first analyzed with method A. With this method, the first tissue compartment is usually assumed to be the free tracer and the second tissue compartment the non specifically bound tracer. However, SA showed that a model with three reversible compartments is the most parsimonious to mathematically describe CER (see Fig. 4.6) in more than 50% of subjects. CER has also been quantified by using methods B and C (see Fig. 4.9 and 4.10). Plots of the predicted kinetics in this region (Fig. 4.16) shows that 2-TM exhibit a high inter-subject variability of the kinetics prediction, while inter-subject variability lower when method B is used. Plots also suggest that the three compartments needed to model CER could be represented by one free pool plus two (one faster and a second one slower) nonspecific uptake compartments or, equally, by a free pool plus a nonspecific compartment and a presence of specific uptake. Recently, Pasey et al. [52] demonstrated the presence of specific binding in the cerebellar vermis and suggested to select the cerebellar white matter (CW) as the new reference region because it improves identifiability and time stability of BP in cortical regions.

In conclusion, method B of [carbonyl $\rightarrow^{11}$ C]WAY-100635 kinetics is accurately identified and provides BP values that are consistent with the known distribution of $5-HT_1A$ receptors in brain, while method A seems unable to
Figure 4.16: Inset graph: Two-tissue model (solid line) and Three-tissue model predictions of the first tissue compartment; Large graph: Two-tissue model (solid line) and Three-tissue predictions for the other compartments.
separately describe the nonspecific and specific binding components. As far as \( BP \) is concerned, method A is underestimating with respect to method B, particularly in regions with high specific binding (HIP and MTC). Finally, CER is well described by a Three-Tissue compartment model.

4.4.3 Compartmen tal Models without Arterial Input Function

4.4.3.1 ROI Studies

Results for the standard Reference Tissue model (RTM) and its simplified version (SRTM) are shown in Table 4.12. Both RTM and SRTM methods give precise estimates of all parameters. \( R_1 \) is \( 0.78 \pm 0.1 \) (mean±SD) for RTM and \( 0.86 \pm 0.06 \) (mean±SD) for SRTM. The problem, though, is not in the estimates precision, but in the heavy under and over estimation of parameters \( k_2 \) and \( BP \) respect to methods B.

\( BP \) is underestimated by both reference tissue models. \( BP \) mean underestimation is between 66% in OCC and 72% in HIP and MTC for RTM and, analogously, it is between 66% in OCC and 73% in MTC for SRTM.

\( k_2 \) is instead more variable. It is very much overestimated by RTM (between 15% in OCC and 119% in ANC) and very much underestimated by SRTM (-73% in MTC to -83% in OCC). This results are confirmatory of those by Parsey et al. [61] in a group of five healthy male volunteers. The cause of this bias is probably the violation of the assumptions on which the models are based. This issue will is further discussed below.

4.4.3.2 Simulation Studies

Simulation Strategy

RTM and SRTM models under simulated conditions were evaluated in order to quantify the extent of BP bias in presence of one or more RTM and SRTM assumption violations. To do so, healthy brain time activity curves (TACs) were synthetized using a representative plasma input function for \([\text{carbonyl } ^{11}C]\)WAY-100635 data and model 2-TM to obtain typ-
### Table 4.12: Compartmen tal models without Arterial Input Function Results

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ical LOF, HIP and OCC ROIs, characterized by a medium, an high and a low receptor density respectively. 1-TM model was also used to obtain a typical CER reference region. The ROI TACs were all generated assuming both different vascular volumes $V_b$ (i.e. equal to 0%, 5% or 10%) and $V_f+ns(ROI) = V_f+ns(1 - TM_{Cer})$ or $V_f+ns(ROI) \neq V_f+ns(1 - TM_{Cer})$ (the normalized respect to CER difference in non specifically bound tracer volumes being $\pm 5\%$, $\pm 20\%$, $35\%$, and $45\%$). Given these synthetic activities, the RTM and the SRTM parameters were identified and compared to their true values. After that, to test the assumption about the reference region tissue compartment model (assumed by RTM and SRTM to be 1-TM), CER activity was generated by using a 2-TM model configuration. Again, the ROI time activity curves were generated assuming both different vascular volumes $V_b$ (i.e. equal to 0%, 5% or 10%) and $V_f+ns(ROI) = V_f+ns(1 - TM_{Cer})$ or $V_f+ns(ROI) \neq V_f+ns(1 - TM_{Cer})$ (the normalized respect to CER difference in volumes being $\pm 5\%$, $\pm 20\%$, $35\%$, an %). To summarize, four cases were considered:

1. case 1, both Reference and ROI are modeled with a 2-TM;

2. case2, Reference is modeled with a 2-TM with almost collapsing compartment (i.e. their compartment kinetics are kinetically indistinguishable) and the ROI is modeled with a 2-TM;

3. case 3, Reference region is modeled with a 1-TM and ROI with a 2-TM (this is the 'ideal' case of the RTM model);

4. case 4, where both Reference region and ROI are modeled as a 1-TM (this is the case of the SRTM model).

RTM and the SRTM models were, then, identified from data and estimated parameters were compared to the true ones. All simulations were performed in MATLAB.

In particular, in the simulation, the arterial plasma $C_p(t)$ curve was derived from the mean plasmatic curve of a 4 healthy young women $[\text{carbonyl}-^{11}\text{C}]\text{WAY-100635}$ data set (see Fig. 4.17) defined over 90 min. The arterial
blood tracer $C_b(t)$ curve was the mean arterial blood curve taken from the 4 subjects $[\text{carbonyl} - ^{11}\text{C}]\text{WAY-100635}$ data set and $V_b$ was assigned each time to different values: 0, 0.05, and 0.1. Rate constants $k_i$ ($i = 1, \ldots, 4$) were first taken from $[\text{carbonyl} - ^{11}\text{C}]\text{WAY-100635}$ healthy young women literature data [61]. Then, in order to keep parameter $BP$ the same while testing the reference tissue hypothesis about the non specifically bound volumes $(V_{f+ns}(ROI) \neq V_{f+ns}(Ref))$ (the difference in non specifically bound tracer volumes being $\alpha = \pm 5\%, \pm 20\%, 35\%$, and 45%), rate constants $K_1$, $k_3$ and $k_4$ were kept equal to their literature values, while rate constant $k_2$ was derived as follows:

$$k_{2ROI} = \frac{K_{1ROI}}{V_{f+ns}(Ref)(1 - \alpha)} \quad (4.54)$$

It is important to notice that $\alpha$ values were chosen in agreement with the two possible physiological interpretation of the 2-TM compartments introduced in the previous section for $[\text{carbonyl} - ^{11}\text{C}]\text{WAY-100635}$ data. Negative $\alpha$s are associated to the classical 2-TM compartments interpretation where the first compartment represents the free + non specific binding and the second compartment represents the specific binding of tracer to target receptors. In this case it is reasonable to have $V_{f+ns}(ROI)$ always equal to or greater than $V_{f+ns}(Ref)$ (i.e. $V_{fCER}$). If we consider, instead, the novel 2-TM compartments interpretation introduced in section 4.4.2 and presented
4. ROI Modeling

In [65] where the first compartment is a free + very small part of non specific pool and the second compartment is a specific + most part of non specific pool, then it is reasonable to have \( V_{f+ns}(ROI) \) always less than or equal \( V_{f+ns}(Ref) \) (i.e. \( V_{ICER} \)). This case is mathematically represented by using positive \( \alpha \).

After having generated all the noise-free TACs some zero mean Gaussian noise was added in order to obtain typical healthy noisy brain time activities. In particular, the Gaussian noise was chosen to have a fractional standard deviation (FSD) larger at the beginning of the experiment (107% at the first time instant and then 11%, 9% 7%, etc.) and decreasing to 3% in the end. 500 different noise generation were considered each time. Given the noisy brain time activity curves (500 for each ROI in each different condition), the second step was to identify the RTM and the SRTM model from this data (i.e. to estimate the models rate constants).

Simulation Results

A first result is that SRTM algorithm always generated parameter estimates whose CV was lower than RTM. Even if the RTM identification algorithm performed well for the HIP region, its performance changed when regions characterized by a lower receptor density were identified. For the LOF region the rejected simulations were only 6% in the worst case, while for the OCC region the rejected simulations were even 75% in the worst case (i.e. case 1, where OCC is modeled by the 2-TM, CER is modeled by the 2-TM, \( V_b = 0 \) and \( V_{f+ns}(OCC) \) is 45% greater then \( V_{ICER} \)).

The Binding Potential

Of interest were also the results on \( BP \). Simulations showed that only if the strict hypothesis of RTM and SRTM model (CER modeled with a 1-TM model, ROI modeled with a 2-TM model, \( V_{f+ns}(ROI) = V_{f+ns}(Ref) \), and \( V_b = 0 \% \) ) hold, then the BP is not biased. As soon as one of RTM and SRTM assumptions fails, BP is biased.

Results for each considered regions of interest are now presented. In Fig. 4.18 the HIP BP % Deviation from the ‘real’ BP value is reported, both as a function of the % deviation of \( V_{f+ns}(CER) \) volume (i.e.\( V_{ICER} \) since the Cere-
4. ROI Modeling

Figure 4.18: HIP BP % Deviation from its true value

bellum is assumed to be devoid of specific receptors) from the \( V_{f+ns}(ROI) \) volume (see \( x \)-axis) and the ROI vascular volume \( V_b \) (assumed to vary from 0, to 5% and 10%, see the solid and dashed lines). The upper graphs represent case 1 (i.e. both the HIP and the CER region modeled with a 2-TM), the lower graphs represent case 3 (i.e. HIP modeled with a 2-TM and CER modeled with a 1-TM). The left side reports the RTM model results and the right side is about the SRTM model. It is evident that when the Cerebellum region is generated with a 2-TM model (see Fig. 4.18 upper graphs) the BP coming from both the RTM and the SRTM model is always underestimated. The higher the \( V_b \) volume the higher the underestimation (even 75% when \( V_{tCER} \) is the 45% higher than the \( V_{f+ns}(HIP) \)); and, the higher the difference in the tracer non specific distribution volumes between CER and HIP region (\( x \)-axis from left to right) the higher the underestimation. When the Reference region TAC is generated assuming a 1-TM (see Fig. 4.18 lower graphs), then the \( BP_{RTM} \) is always biased except when \( V_b = 0\% \) and the CER and the HIP region have the same non specific distribution volumes. Even if the estimated \( BP_{SRTM} \) is never equal to its true value, when \( V_b = 0\% \) and the non specific distribution volumes between CER and HIP are the same,
then the overestimation is only 9%. 

In Fig. 4.19 the OCC BP % Deviation from the 'real' BP value is reported. Again, the upper graphs represent case 1 (i.e. both the OCC and the CER region modeled with a 2-TM), the lower graphs represent case 3 (i.e. OCC modeled with a 2-TM and CER modeled with a 1-TM). The left side reports the RTM model results and the right side is about the SRTM model. Again (see Fig. 4.19 upper graphs), when the CER TAC is generated assuming a 2-TM model the BP is biased. The OCC curves seem to be shifted toward the upper y-axis compared to the HIP curves and only when \( V_{\text{CER}} \) is ~15% lower than \( V_{f+ns(OCC)} \) and \( V_b = 0\% \) the BP is correctly identified from the RTM and the SRTM model. Again, as the \( V_b \) volume becomes higher the BP % Deviation curves are shifted downwards; and, as the difference in the tracer non-specific distribution volumes goes from negative to positive values (x-axis from left to right) the three curves follow a linear decreasing straight line. If the CER TAC is generated assuming a 1-TM (see Fig. 4.19 lower graphs), then the \( BP_{RTM} \) is always biased except when \( V_b = 0\% \) and the CER and the OCC region have the same non-specific distribution volumes. In this case, the \( BP_{SRTM} \) overestimation is quite small,
4. ROI Modeling

only 3%.

Graphs of the LOF $BP\%$ Deviations from its true value are not reported. Anyway this region results are quite similar to that of the OCC region. Again, when the CER TAC is generated assuming a 2-TM the LOF curves seem to be shifted toward the upper y-axis compared to the HIP curves, but less than the OCC curves. Moreover, only when $V_{ICER}$ is about 30% lower than $V_{f+ns}(LOF)$ and $V_b = 0\%$ the $BP$ is correctly identified from the RTM and the SRTM model. Otherwise it is over- and underestimated. (x-axis from left to right). If the CER TAC is generated assuming a 1-TM, then the $BP_{RTM}$ is always biased except when $V_b = 0\%$ and the CER and the LOF region have the same non specific distribution volumes. In this case, the $BP_{SRTM}$ overestimation is about 5%.

Simulation analysis also showed that only if the regional time activity curves are generated as assumed by RTM (CER 1-TM, ROIs 2-TM, $V_b = 0\%$ and $V_{ICER} = V_{f+ns}(ROI)$), then $BP$ estimated by RTM differs from the one calculated by the SRTM model. As soon as one or more of the RTM and SRTM model assumption failed we always found $BP_{RTM} = BP_{SRTM}$. Here are shown tables representing the mean (mean ± SD) $BP_{SRTM}\%$ Deviation from the $BP_{RTM}$ (see Table 4.13) calculated respect to the difference in non specific volumes between ROIs and CER. As always, upper graphs represent case 1 (i.e. both the ROI and the CER region modeled with a 2-TM), and the lower graphs represent case 3 (i.e. ROIs modeled with a 2-TM and CER modeled with a 1-TM). It is possible to notice that, independently from the difference in non specific volumes between ROIs and CER, when the TACs are generated assuming a 2-TM for the CER region (upper table), then the mean $BP_{SRTM}\%$ deviation from the estimated $BP_{RTM}$ is within ±1.5% for the three regions. In particular, for the HIP region it is almost 1.5% when $V_b$ is larger than 0%, and for the other regions it is almost 0%. When the CER region is instead modeled with a 1-TM (see lower table), then the % deviation from the two reference models is still small but more visible. And it is even possible to notice that for 'high regions', i.e. characterized by an high receptor density, this deviation is even more evident, while 'intermediate' or 'low' region are characterized by a lower $BP\%$ Deviation (see Table 4.13,
4. ROI Modeling

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<td>Occ</td>
<td>-0.04 ± 0.3</td>
<td>0.1 ± 0.1</td>
<td>0.2 ± 0.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ROI</th>
<th>$V_b=0$</th>
<th>$V_b=0.05$</th>
<th>$V_b=0.1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hip</td>
<td>mean ± SD</td>
<td>mean ± SD</td>
<td>mean ± SD</td>
</tr>
<tr>
<td>9 ± 0.7</td>
<td>7 ± 0.4</td>
<td>5 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Lof</td>
<td>5 ± 0.7</td>
<td>5 ± 0.5</td>
<td>4 ± 0.5</td>
</tr>
<tr>
<td>Occ</td>
<td>4 ± 0.6</td>
<td>3 ± 0.6</td>
<td>2 ± 0.5</td>
</tr>
</tbody>
</table>

Table 4.13: $BP_{SRTM}$% Deviation from the $BP_{RTM}$. Upper Table is case 1 and lower Table is case 3.

Cases 2 and 4, whose results were not reported for space saving problems, need also to be mentioned. Case 2 concerns about the CER time activity curves generated as if the two tissue compartment were indistinguishable from a kinetic point of view (the ROI still being modeled with a 2-TM). Case 4 concerns about both the ROI and CER TACs generated assuming a 1-TM. Results from our simulation in this two cases are in agreement with the ones showed in this paper for cases 1 and 3. For case 4 we found $BP_{RTM} = BP_{SRTM}$. This is in agreement with our hypothesis that when the RTM assumptions fail, $BP$ parameter estimation is corrupted. We found the mean $BP_{SRTM}$% deviation from the estimated $BP_{RTM}$ to vary from 0.1% to 0.01% in the OCC region, from 0.59% to 0.31% in the LOF region and from 1.8% to 1.12% for region HIP (tables not shown). For case 2 things are different. In this case the CER time activity curve is generated assuming a 2-TM, even though from the 'outside' only one tissue compartment is visible. We are closer to the model assumption of the Reference Tissue model and, in fact, we found that the mean $BP_{SRTM}$% deviation from $BP_{SRTM}$ was growing from the almost 0% of case 1 to the case 3 values (see Table 4.14). Therefore, for what concerns $BP$, as long as the TACs are generated according to RTM...
and SRTM model assumptions, than the Binding Potentials estimated by the two methods tend to differentiate.

**Parameter R1**

Simulations indicated that $R_1$ parameter is quite well estimated in every situation. If HIP region is considered, in case 1 the mean overestimation (calculated respect to the $V_{icER}$ % Deviation from $V_{f+ns}(HIP)$) was between 3% (when $V_b = 0%$) and 11% (when $V_b = 10%$) for the RTM model and between 5% ($V_b = 0%$) and 19% ($V_b = 10%$) for the SRTM model. In case 3 the mean bias was between -4% ($V_b = 0%$) and 7% ($V_b = 10%$) for the RTM model, and between -11% ($V_b = 0%$) and 0% ($V_b = 10%$) for the SRTM model.

For what concerns OCC region, the mean overestimation in case 1 was found to vary between 6% ($V_b = 0%$) and 13% ($V_b = 10%$) for the RTM model and between 2% ($V_b = 0%$) and 7% ($V_b = 10%$) for the SRTM model. In case 3 R1 was always underestimated. The mean underestimation was between 6% ($V_b = 0%$) and 2% ($V_b = 10%$) for the RTM model and between 15% ($V_b = 0%$) and 10% ($V_b = 10%$) for the SRTM model.

LOF region results are quite similar. In case 1 the mean overestimation varied between 4% ($V_b = 0%$) and 10% ($V_b = 10%$) for the RTM model and between 2% ($V_b = 0%$) and 9% ($V_b = 10%$) for the SRTM model. In case 3 the mean bias was between -5% ($V_b = 0%$) and 1% ($V_b = 10%$) for the RTM model and between -14% ($V_b = 0%$) and -7% ($V_b = 10%$) for the SRTM model.

**Parameter $k_2$**

Very different results were found for rate constant $k_2$. $k_2$ can be heavily

<table>
<thead>
<tr>
<th>ROI</th>
<th>$V_b=0$</th>
<th>$V_b=0.05$</th>
<th>$V_b=0.1$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean ± SD</td>
<td>mean ± SD</td>
<td>mean ± SD</td>
</tr>
<tr>
<td>Hip</td>
<td>7 ± 2.6</td>
<td>7 ± 1.8</td>
<td>5 ± 1.8</td>
</tr>
<tr>
<td>Lof</td>
<td>4 ± 1.6</td>
<td>4 ± 1.4</td>
<td>3 ± 1.4</td>
</tr>
<tr>
<td>Occ</td>
<td>3 ± 1.5</td>
<td>3 ± 1.4</td>
<td>2 ± 1.3</td>
</tr>
</tbody>
</table>

Table 4.14: $BP_{SRTM}$% deviation from the $BP_{RTM}$, case 2
4. ROI Modeling

Figure 4.20: HIP $k_2$ % Deviation from its true value

overestimated (even 550%) by the RTM model or it can be very underestimated by the SRTM model (~70% or more). In the following graphs is reported $k_2$% Deviation from its true value as identified from the RTM and SRTM models in region HIP (Fig. 4.20) and OCC (see Fig. 4.21). Taking a look to Fig. 4.20 it is evident that what influences more this parameter estimation is not $V_b$ but the Reference region compartmental structure (1-TM better than 2-TM) and the difference in Non specific volumes. In the hypothesis of the RTM model ($V_b = 0$, $V_{ICER} = V_{f+ns}(HIP)$ and CER modeled with a 1-TM) $k_2$underestimation is 12%. When $k_2$ is estimated by using the SRTM model, its % Deviation from the true value varies from about 40% (CER 2-TM, $V_b = 0$% and $V_{f+ns}(HIP)$ 45% greater than the $V_{ICER}$) to about -7% (CER 1-TM, $V_b = 0$% and $V_{f+ns}(HIP)$ 45% greater than the $V_{ICER}$). When $V_b = 0$, $V_{ICER} = V_{f+ns}(HIP)$ and CER modeled with a 1-TM, $k_2$SRTM underestimation is ~ 46%.

Things are going even worse for the OCC region (see Fig. 4.21). Again, what seems to heavily influence the $k_2$ estimation is the Reference region model (1-TM does better than 2-TM) and the difference in non specific volumes, even if now, when $V_b$ is equal to 10%, $k_2$ deviation from its true value
4. ROI Modeling

for the RTM model is even more evident (especially in the central portion of the graph, see lower left graph). When, in case 1, BP is correctly estimated (i.e. when $V_{ICER}$ is $\sim 15\%$ lower than $V_{f+ns}(OCC)$, and $V_b = 0\%$), then $k_2$ % Deviation from its true value is extremely high: it is about 1750 %. In the hypothesis of the RTM model ($V_b = 0\%$, $V_{ICER} = V_{f+ns}(OCC)$ and CER modeled with a 1-TM) $k_2$ underestimation is only 4%. When $k_2$ is estimated by using the SRTM model, its % deviation from the true value varies from about 25% (CER 2-TM, $V_b = 0\%$ and $V_{f+ns}(OCC)$) to about -20% (CER 1-TM, $V_b = 0\%$ and $V_{f+ns}(OCC)$ 45% greater than the $V_{ICER}$). When, in case 1, BP is correctly estimated (i.e. when $V_{ICER}$ is $\sim 15\%$ lower than $V_{f+ns}(OCC)$, and $V_b = 0\%$), then $k_{2SRTM}$ % Deviation from its true value is only 2%. When $V_b = 0\%$, $V_{ICER} = V_{f+ns}(OCC)$ and CER modeled with a 1-TM, $k_{2SRTM}$ underestimation is 56%.

Region LOF has a similar behavior, much similar to OCC than HIP. An interesting difference from the OCC region is that the LOF region seems to be less dependent on the $V_b$ value. The $V_b = 10\%$ curve in the left lower graph is closer to the other curves in the LOF graph.

Parameter $k_3$ and $k_4$
Rate constants $k_3$ and $k_4$ % deviations from their true value have a more complicated shape than $k_2$. See results about the HIP region in Fig. 4.22. The upper graphs represent case 1 (i.e., both the HIP and the CER region modeled with a 2-TM), and the lower graphs represent case 3 (i.e., HIP modeled with a 2-TM and CER modeled with a 1-TM). The left side reports the $k_3$ results and the right side is about the $k_4$ estimated parameters. When the Reference and the HIP region are generated assuming a 2-TM (see Fig. 4.22 upper graphs), $k_3$ % deviation from its true value has a quite linear shape. $k_3$ is always underestimated, more when $V_b$ is higher and $V_{TCP}$ is greater than $V_{f+n}(ROI)$. In this situation rate constants $k_4$ is almost always overestimated (except when $V_{TCP}$ is about 30% lower than $V_{f+n}(ROI)$) and the overestimation is reduced when $V_b$ is 5% or 10%. $k_4$ curves have quite a linear shape too. Things change when the TACs are generated assuming for CER a 1-TM (see Fig. 4.22 lower graphs). $k_3$ curves ($V_b=0, 5, 10\%$) don’t have a linear-like shape anymore and the estimation process is heavily dependent on $V_b$. When $V_b$ is 0% the curves has already lost its linear-like shape, and when $V_b$ becomes greater, things are even worse. The same can be said for rate constants $k_4$, even though its % deviation curves seems to still have
a more linear shape. Things start going worse when \( V_b \) becomes greater than 5\%. It is important to notice that, even though here only \( k_3 \) and \( k_4 \) results for region HIP are represented, the same results were found for region OCC and LOF. Moreover, \( k_3 \) and \( k_4 \) % Deviation curves were even more variable for these two latter regions when CER was modeled by a 1-TM.

4.4.4 Discussion

4.4.4.1 ROI Study Discussion

Spectral analysis suggested the presence of three tissue compartments in regions except HIP and DRN. A possible explanation could be that these high uptake regions probably have a specific binding component that is predominant, so that the non specific spectral component is present but cannot be reliably separated. Of note is that the selection of the three-exponential model was never found in literature before. Therefore, in order to verify this result, the possibility that a different model used for the unchanged tracer fraction (UF) during metabolite correction could affect the shape of the arterial curve corrected for the presence of metabolites and SA results was considered.

The analysis performed on 4 subjects proved that the model used to obtain \([\text{carbonyl} ^{11}\text{C}]\text{WAY-100635 metabolite-corrected plasma activity curves has a potential impact on the quantification of the spectral components if the difference between 0–5 min AUC} \text{C}_{pLin} \text{ and } C_{pExp} \text{ is greater than 30\% (see section 4.4.1). Results obtained after rerunning SA on the entire dataset with both } C_{pLin} \text{ and } C_{pExp} \text{ confirmed, however, the three-exponential model as the most parsimonious in all ROIs, except HIP and DRN, regardless of UF model.}

Based on this result, three compartmental models with arterial input function were compared. Parameter estimation process was successful for all methods in all regions. In particular, focus was posed on methods B and C, both based on a 3-TM model respectively without and with Bayesian constraints. In agreement with SA results, method B, the standard 3-TM, gave better estimates of all parameters in terms of AIC, fits and parameter preci-
4. ROI Modeling

...sion in all regions except HIP and DRN, where a 2-TM model was selected as the model of choice for \( \text{[carbonyl}^{\text{\text{11}}\text{C}]WAY-100635} \) quantification.

Method C, the constrained 3-TM model, always gave lower AICs than method B in all regions except HIP and DRN where it improved the convergence rate. In HIP and DRN, anyway, this method performed worse than method A and therefore was discharged.

For what concerns the arterial compartmental models, another result is that the standard 2-TM model is characterized by a kinetics that does not follow the classic interpretation (i.e. first tissue compartment = free + non specific uptake and second tissue compartment = specific uptake) as in Fig. 4.2. By plotting the compartmental kinetics predicted by methods A and B it was evident that a new physiological interpretation of the compartments in the 2-TM model is called for: the first tissue compartment represents mainly free kinetics and the second one is a combination of specific and part of the nonspecific components (see Fig. 4.14 and 4.15).

Particular attention is required for region CER, where a 3-TM model is resolvable in 6 of the 9 subjects with good CVs. CER was quantified by using methods A, B and C (see Fig. 4.9 and 4.10). Plots of the predicted kinetics (method A and B) in this region (Fig. 4.16) shows that 2-TM exhibit a high inter-subject variability of the kinetics prediction, while inter-subject variability lowers when method B is used. Recently, Parsey et al. [52] demonstrated the presence of specific binding in the Cerebellum that can explain these results and the use of a 3-TM model for \( \text{[carbonyl}^{\text{\text{11}}\text{C}]WAY-100635} \) quantification in this region. Care needs to be posed, anyway, on the use of this region as a reference in ligand quantification without arterial input function. A possible strategy for the future could be that proposed by Parsey et al. [52] who suggested to select as a reference regions a subregion of CER devoid of specific receptors (cerebellar white matter).

Finally, results with standard compartmental modeling without arterial input function (RTM and SRTM) were disappointing. In fact, both RTM and SRTM produced heavy under and overestimation of parameters \( k_2 \) and \( BP \) respect to the same values from method B.
4. ROI Modeling

4.4.4.2 Simulation Study Discussion

In order to understand and quantify the extent of bias in presence of one or more RTM and SRTM assumption violations, methods RTM and SRTM were evaluated under simulated conditions.

Simulations showed that a correct estimation of the binding potential with RTM and SRTM models is possible only if all the assumption underlying these models are verified. As soon as one of them fails, $BP$ and the other RTM and SRTM parameters are biased.

Simulation analysis showed that (see Fig. 4.18 and 4.19), both an under or overestimation of $BP$ is possible, even if the previous analysis reported in this study on real $[\text{carbonyl} - ^{11}\text{C}]\text{WAY}-100635$ data always evidenced a $BP$ underestimation for both RTM and SRTM models. Other groups [57, 43, 61] have always reported the same $BP$ underestimation when applying RTM and SRTM. Anyway, the result obtained by simulation is not in contrast with their results. The Simulation analysis presented in this study, in fact, is a generalization of previous studies and a variety of different conditions are tested.

Simulations also showed that RTM and SRTM $BP$ bias is marked in high density receptor region (HIP) while relatively modest in intermediate and low density ROIs (LOF and OCC). This is a major concern because a systematic correction of $BP$ values is not possible. Similar results were reported by Slifstein et al. [43](see Fig. 2 in [43], where the regression curve of the $BP_{SRTM}$ to the $BP_{2-TM}$ had a slope less than unity and a y intercept greater than zero). $BP$ bias is caused by the violation of RTM and SRTM model hypotheses, and results indicate that each one of this hypotheses, if violated, increases $BP$ bias and it is difficult to understand which one of them gives the greater contribution.

In particular, then, when the hypothesis on CER model is violated (i.e. CER is modeled by a 2-TM) $BP$ is heavily biased and the estimated $BP$ is the same for RTM and SRTM models. For what concerns the other parameters, $R_1$ is precisely estimated while $k_2$ is far off. Rate constants $k_3$ and $k_4$ are also heavily influenced by CER model structure. When CER region is generated
assuming a 2-TM model $k_3$ and $k_4$ maintain their linear like shape and they loose it when CER is generated assuming a 1-TM. It is of note, though, that simulation results reported in this study are not in disagreement with Slifstein et al. [43](see Table 2). In fact, the analysis presented here is a generalization of Slifstein work, where $V_b$ equal to 0% and $V_{iCER}$ equal to $V_{f+ns}(ROI)$ are considered.

The violation of the hypothesis on the different hematic volume within the ROI also influences the parameters estimation, while no influence of $V_b$ on the $BP_{SRTM}$ % deviation from $BP_{RTM}$ is found. The more the $V_b$ within the ROI, the lower the $BP$ curves on the graphs, no matter which compartmental structure is associated to the CER region (1-TM or 2-TM). Results regarding the positive part of the x-axis in Fig. 4.18 and 4.21 are in agreement with Gunn et al. [57] where a sensitivity of the binding potential to the blood volume component (see [57] Fig. 6) is shown. For what concerns the estimated parameters, $R_1$ is dependent on the $V_b$ value even if it is always precisely estimated. Rate constants $k_2$, then, is dependent on the blood Volume $V_b$ inside the ROI, even if no dependence on the CER structure (1-TM vs 2-TM) or the two identification models RTM and SRTM can be found. When CER is modeled by a 1-TM, rate constants $k_3$ and $k_4$ become more dependent on the blood volume within the ROI and their curves become more variable (less linear) as soon as $V_b$ increases, (see Fig. 4.22).

Finally, the hypothesis on the equal volume distribution of the not specifically bound tracer between reference and ROI is crucial for a good parameter estimation. Going from left to right in the $BP$ and $k_2$ graphs it is possible to notice that the parameters curves follow a decreasing linear shape, regardless of CER model structure (1-TM or 2-TM). Things are even more complicated for rate constants $k_3$ and $k_4$(see Fig. 4.22).

Simulations also points out the fact that only when the hypothesis on the reference region model is valid, i.e. the CER region described by two collapsing compartment (case 2) or by a single compartment (case 3), and the ROI is modeled with a 2-TM, the $BP_{SRTM}$ differentiate from the $BP_{RTM}$ (see Tables 4.13 and 4.13). Further analysis is, indeed, needed to try to understand if this result has a general validity. If this was the case, then we
4. ROI Modeling

could have a general method (not dependent on the selected tracer) that could guide us in the understanding of a regional compartmental model structure given the ROI time activity curve. We could apply, for example, RTM and SRTM model to the ROI data and compare the two outcome BP values. If they were different, then the compartmental structure of the CER and the ROI under study would be equal to the one assumed by the reference tissue theory; otherwise (i.e. if BPs were equal) the compartmental structure would probably be either more simple (CER 1-TM and ROI 1-TM) or more complex (CER 2-TM and ROI 2-TM).

To summarize, our simulation showed that a correct estimation of the binding potential with RTM and SRTM models is possible only if all the assumption underlying these models are verified. As soon as one of them fails, BP and the other RTM and SRTM parameters are biased. Unfortunately, it is not possible to find a unique cause of the RTM and SRTM BP bias. For sure, the hypothesis on the Reference region (CER) model is crucial for the correct estimation of the important parameters, even if the extent of the bias also depends on the considered region of interest, on the $V_b$ value and on the real difference in the not specifically bound tracer volumes between the reference and the ROI.

In conclusion, in presence of the arterial plasma curve, it is possible to conclude that the best model for $[^{11}\text{C}]\text{WAY-100635}$ kinetics quantification of young healthy women is model B (i.e. the standard 3-TM), since this method converged quite well in all regions except HIP and DRN giving good fits, precise estimates of parameters and lower AIC values respect to the other arterial methods. In regions HIP and DRN the model of choice is 2-TM, probably because the specific binding component, in these two region, is very high, and prevents the separation of the non specific and free ones. If the arterial curve is not available, the reference tissue models should be used with utmost care and the bias described in this study should be kept in mind in the design and interpretation of studies performed with $[^{11}\text{C}]\text{WAY-100635}$ using RTM and SRTM models.
Chapter 5

Pixel By Pixel Modeling

Positron Emission Tomography quantification can be performed at ROI levels and pixel level. The advantages of the pixel approach with respect to the ROI approach is the possibility of generating parametric images of tracer binding without any loss of spatial resolution. Two of the most popular methods used for parametric imaging are the conventional standard Logan graphical method (presented in chapter 4 for ROI analysis) and the simplified reference tissue method (SRTM) as implemented by Gunn et al. [59]. Both methods require linear fitting of parameters, which is necessary because the high noise level of pixel time activity curves prevents the identification with nonlinear estimation methods like weighted nonlinear least squares (WNLLS).

In this chapter the most promising parametric imaging methods found in literature are presented and results relative to $[^{11}\text{C}]-\text{DASB}$ imaging are discussed.

5.1 Methods

5.1.1 The Generalized Linear Least Squares

The Generalized Linear Least Squares (GLLS) method, with and without weights, was applied to PET dynamic data as implemented in Feng et al. in 1996 [9]. The main idea under this method is to start generating parametric images by using Linear Least Squares (LLS) estimation and then to correct
5. Pixel By Pixel Modeling

the bias, obviously associated to the generated image, by applying a filter to that image.

Assuming that the \( n \)th order differential equation describing a general SISO linear continuous dynamic system is:

\[
y^{(n)}(t) + a_1 y^{(n-1)}(t) + \ldots + a_n y(t) = b_1 u^{(n-1)}(t) + \ldots + b_n u(t) \tag{5.1}
\]

where \( u(t) \) is the system input (the plasmatic curve \( C_p(t) \)) and \( y(t) \) is the system output (\( C_i(t) \), the tracer radioactivity in tissue), and \( a_1, \ldots, a_n \) and \( b_1, \ldots, b_n \) are the system transfer function parameters, the Laplace transform of the above equation is:

\[
[s^n Y(s) - s^{n-1} y(0) - \ldots - y^{(n-1)}(0)] + a_1 [s^{n-1} Y(s) - \ldots - y^{(n-2)}(0)] + \ldots + a_n Y(s) = \[b_1 [s^{n-1} U(s) - \ldots - u^{(n-2)}(0)] + \ldots + b_n U(s) \tag{5.2}
\]

where \( u(0), \ldots, u^{(n-2)}(0) \) and \( y(0), \ldots, y^{(n-1)}(0) \) are the initial conditions for the input and output functions. Equation 5.2 can be rearranged in:

\[
(s^n + a_1 s^{n-1} + \ldots + a_n)Y(s) = (b_1 s^{n-1} + \ldots + b_n)U(s) + ic(s).
\]

\[
A(s)Y(s) = B(s)U(s) + IC(s) \tag{5.3}
\]

where \( IC(s) \) is a term of linear combinations of the input and output initial conditions. In most cases, initial conditions are all zeros, and dividing both sides of equation 5.3 by \( s^n \) and rearranging we get:

\[
Y(s) = -a_1 s^{-1} Y(s) - \ldots - a_n s^{-n} Y(s) + b_1 s^{-1} U(s) + \ldots + b_n s^{-n} U(s) \tag{5.4}
\]

Taking the inverse Laplace transform and given \( m \), the total number of time samples, equation 5.4 can be expressed as:

\[
y(t) = C_i(t) = X \theta \tag{5.5}
\]

In this case equation 5.5 represents the relation between \( C_i(t) \) and \( C_p(t) \) of a compartmental model, \( \theta \) is a column vector whose elements are combinations of unknown rate constants \( K_1, K_2, \text{ etc.} \) and \( X \) is the coefficient matrix containing integrations of the output and of the input, or functions of \( t \) as
shown in equation 5.6:

\[
X = \begin{bmatrix}
\int_{0}^{t_1} y(t)dt & \cdots & \int_{0}^{t_1} \int_{0}^{t_1} y(t)dt & \cdots & \int_{0}^{t_1} C_y(t)dt & \cdots & \int_{0}^{t_1} \int_{0}^{t_1} C_y(t)dt & \int_{0}^{t_1} C_y(t)dt & \cdots & \int_{0}^{t_1} \int_{0}^{t_1} C_y(t)dt \\
\int_{0}^{t_2} y(t)dt & \cdots & \int_{0}^{t_2} \int_{0}^{t_2} y(t)dt & \cdots & \int_{0}^{t_2} C_y(t)dt & \cdots & \int_{0}^{t_2} \int_{0}^{t_2} C_y(t)dt & \int_{0}^{t_2} C_y(t)dt & \cdots & \int_{0}^{t_2} \int_{0}^{t_2} C_y(t)dt \\
\vdots & \ddots & \vdots & \ddots & \vdots & \ddots & \vdots & \vdots & \ddots & \vdots \\
\int_{0}^{t_m} y(t)dt & \cdots & \int_{0}^{t_m} \int_{0}^{t_m} y(t)dt & \cdots & \int_{0}^{t_m} C_y(t)dt & \cdots & \int_{0}^{t_m} \int_{0}^{t_m} C_y(t)dt & \int_{0}^{t_m} C_y(t)dt & \cdots & \int_{0}^{t_m} \int_{0}^{t_m} C_y(t)dt \\
\end{bmatrix}
\]

(5.6)

In particular, if the tracer kinetics in a pixel is well described by a 1-TM model, matrix \(X\) becomes:

\[
X = \begin{bmatrix}
-\int_{0}^{t_1} y(t)dt & \int_{0}^{t_1} C_y(t)dt \\
-\int_{0}^{t_2} y(t)dt & \int_{0}^{t_2} C_y(t)dt \\
\vdots & \vdots \\
-\int_{0}^{t_m} y(t)dt & \int_{0}^{t_m} C_y(t)dt \\
\end{bmatrix}
\]

(5.7)

Due to the measurement noise in \(y(t) = C_i(t)\), the left and right side of equation 5.5 may not equal exactly. A more accurate expression should be:

\[
y(t) = C_i(t) = X\theta + \xi \\
Y(s) = \frac{B(s)}{\hat{A}(s)} + E(s)
\]

(5.8)

where \(\xi\) is a column vector form of the equation noise term. Equation 5.5 represents a model that is linear in the parameters, and if the standard LLS algorithm is applied, vector of unknown parameters \(\theta\) can be estimated as:

\[
\hat{\theta}_{LLS} = (X^TX)^{-1}X^Ty
\]

(5.9)

where \(\hat{\theta}_{LLS}\) represents the estimated \(\theta\) in the LLS sense. Anyway, the estimates from 5.9 are biased. This because the noise term \(\xi\) in 5.8 not only contains the direct measurement noise of \(y(t)\) on the left side of 5.8, but also contains linear combinations of the integrations of these measurement errors in the X matrix on the right side of 5.5. In other words, the equation noise \(\xi\) is colored. To whiten the equation noise so that an unbiased estimate of \(\theta\) can be achieved, if a rough value for vector \(\hat{\theta}\) is obtained from 5.9, \(\hat{\theta}_{LLS}\), the
5. Pixel By Pixel Modeling

estimated $A(s)$, can be determined:

$$\hat{A}(s) = s^n + \hat{a}_1 s^{n-1} + \cdots + \hat{a}_n$$  \hspace{1cm} (5.10)

where $\hat{a}_1, \hat{a}_2, ..., \hat{a}_n$ are estimates of $a_1, a_2, ..., a_n$. Dividing 5.8 by $\hat{A}(s)$ one gets:

$$\frac{A(s)Y(s)}{\hat{A}(s)} = \frac{B(s)}{\hat{A}(s)} + \frac{A(s)}{\hat{A}(s)}E(s)$$  \hspace{1cm} (5.11)

If $\hat{A}(s) \rightarrow A(s)$, the equation noise is whitened and the estimates become unbiased. Applying that filter means rearranging the model equation into:

$$r = Z\theta$$  \hspace{1cm} (5.12)

where $Z$ is:

$$Z = \begin{bmatrix}
\sum_{i=1}^{n} \lambda_i \lambda_i^{-1} y_i(t_1) & \cdots & \sum_{i=1}^{n} \lambda_i \lambda_i^{-1} y_i(t_1) & \cdots & \sum_{i=1}^{n} \frac{\lambda_i}{A'(\lambda_i)} & \cdots & \sum_{i=1}^{n} \frac{\lambda_i}{A'(\lambda_i)} \\
\sum_{i=1}^{n} \lambda_i \lambda_i^{-1} y_i(t_2) & \cdots & \sum_{i=1}^{n} \lambda_i \lambda_i^{-1} y_i(t_2) & \cdots & \sum_{i=1}^{n} \frac{\lambda_i}{A'(\lambda_i)} & \cdots & \sum_{i=1}^{n} \frac{\lambda_i}{A'(\lambda_i)} \\
\vdots & \ddots & \ddots & \ddots & \ddots & \ddots & \ddots \\
\vdots & \ddots & \ddots & \ddots & \ddots & \ddots & \ddots \\
\sum_{i=1}^{n} \lambda_i \lambda_i^{-1} y_i(t_m) & \cdots & \sum_{i=1}^{n} \lambda_i \lambda_i^{-1} y_i(t_m) & \cdots & \sum_{i=1}^{n} \frac{\lambda_i}{A'(\lambda_i)} & \cdots & \sum_{i=1}^{n} \frac{\lambda_i}{A'(\lambda_i)}
\end{bmatrix}$$  \hspace{1cm} (5.13)

and $r$ is:

$$r = \begin{bmatrix}
y(t_1) - \sum_{j=1}^{n} a_j \sum_{i=1}^{n} \lambda_i^{n-j} y_i(t_1) & \cdots & y(t_2) - \sum_{j=1}^{n} a_j \sum_{i=1}^{n} \lambda_i^{n-j} y_i(t_2) & \cdots & y(t_m) - \sum_{j=1}^{n} a_j \sum_{i=1}^{n} \lambda_i^{n-j} y_i(t_m)
\end{bmatrix}^T$$  \hspace{1cm} (5.14)

where $\lambda_i$ is the i-th nonzero root of $\hat{A}(s)$.

When $\hat{A}(s) \rightarrow A(s)$, the noise approaches the original white measurement noise. The GLLS estimator is, therefore:

$$\hat{\theta}_{GLLS} = (Z^T Z)^{-1} Z^T y$$  \hspace{1cm} (5.15)

where $\hat{\theta}_{GLLS}$ represents the estimates of $\theta$ in the GLLS sense, the initial $\hat{A}(s)$ provided by 5.9. Theoretically, equation 5.15 needs to be used iteratively.
until \( \hat{A}(s) \rightarrow A(s) \). The termination criterion could be \( \| \Delta \theta \|^2 < \zeta \), where \( \| \Delta \theta \| \) is the Euclidean norm of difference of estimated parameters between two successive iterations.

Weights can also be included in the estimation process and make the quantification of the estimated parameters precision possible. The assumption on the measure noise statistics is that the noise has zero mean and a covariance matrix described by:

\[
\Sigma_v = \sigma^2 B
\]

(5.16)

where \( \sigma^2 \) is a scalar and \( B \) is a diagonal matrix. \( \Sigma_v \)'s elements are assumed to be equal to the inverse of the scan interval between two following acquisitions. When weights are considered, equations 5.9 and 5.15 become:

\[
\hat{\theta}_{WLLS} = (X^T \Sigma_v^{-1} X)^{-1} X^T \Sigma_v^{-1} y
\]

\[
\hat{\theta}_{GWLLS} = (Z^T \Sigma_v^{-1} Z)^{-1} Z^T \Sigma_v^{-1} y
\]

(5.17)

5.1.2 The Ridge Regression with Spatial Constraint (Simple and Generalized)

Ridge regression has been used in statistics to reduce estimation variability of linear regressions at the expense of a small bias in estimates, with the amount of bias dependent on the noise level of data [17, 18]. Also, depending on whether the magnitude of different parameters are scaled to the same level or not, there are “generalized” (GRRSC) and “simple” (SRRSC) ridge regressions. The use of ridge regression with spatial constraint for generating parametric images from dynamic PET studies has recently been explored by Zhou et al. 2001 [73]. This method improves the signal-to-noise ratio of parametric images obtained applying LLS identification.

As discussed in the previous section, parametric images estimated by LLS are unsatisfactory. In order to reduce their noise, the general ridge regression with spatial constraint method requires two steps: first, parametric images obtained by LLS identification are spatially smoothed; second, ridge regression is applied using the smoothed parametric images obtained in the
first step as constraints. In the first step, after LLS application to each pixel of a PET dynamic, the resulted parametric image $\beta$ is spatially smoothed with a filter $S$, and the smoothed $\beta$ is denoted as $\beta_s$. The noise variance of the data ($\sigma^2$) for each pixel is estimated a posteriori from the residuals of the LLS. Based on the estimates of $\sigma^2$ and $\beta$, the diagonal ridge matrix $H$ is calculated as:

$$h_i = \frac{\sigma^2}{2(\beta_i - \beta_s)} \quad 1 \leq i \leq m \quad \text{GRRS}$$

$$h_i = \frac{\sigma^2}{(\beta_i - \beta_s)^T(\beta_i - \beta_s)} \quad 1 \leq i \leq m \quad \text{SRRSC} \quad (5.18)$$

$h_i$ is then also smoothed spatially by filter $S$.

The second step applies the ridge regression. As shown in [73] ridge regression is equivalent to minimizing a cost function that has the form:

$$Q(\beta/H) = (Y - X\beta)^T W(Y - X\beta) + (\beta - \beta_s)^T H(\beta - \beta_s) \quad (5.19)$$

where $Y$ is a measured time activity vector, $X$ is the regression coefficient matrix determined by the tracer kinetic model, $W$ is a diagonal matrix whose elements are equal to the duration of the $i^{th}$ frame of a PET dynamic scanning. As can be seen in equation 5.19, the cost function for SRRSC and GRRSC includes an additional penalty term. Since matrix $H$ is proportional to the noise variance of the measured data, the penalty term automatically adjusts for the noise level of the pixel kinetics.

If 5.19 is converted to a centralized form by letting:

$$\beta_1 = \beta - \beta_s$$
$$Y_1 = W_1 Y$$
$$X_1 = W_1 X \quad (5.20)$$

where $W = \text{diag}(w_{ii})^{0.5}$, then the cost function to be minimized becomes:

$$Q(\beta_1/H) = (Y_1 - X_1\beta_1)^T(Y_1 - X_1\beta_1) + \beta_1^T H \beta_1 \quad (5.21)$$

and the solution that minimizes the above cost function can be determined.
to be:

$$\beta(H) = (X^TWX + H)^{-1} (X^TWX + H\beta_s) \quad (5.22)$$

For SRRSC, it can be further simplified to:

$$\beta(H) = (X^TWX + hI_m)^{-1} (X^TWX + h\beta_s) \quad (5.23)$$

where $m$ is the dimension of parameter vector $\beta$ and $I_m$ is an $m$-dimensional identity matrix.

It can be seen from the above equations that as $H$ or $h$ tends to zero, GRRSC/SRRSC becomes regular LLS. As $h$ or the minimum diagonal elements of $H$ tends to infinity, the results of GRRSC/SRRSC will be $\beta_s$, and the bias introduced by GRRSC/SRRSC is limited by the spatial constrain $\beta_s$.

### 5.1.3 The Multilinear Reference Tissue Models

A Multilinear Reference Tissue method proposed by Ichise et al. [38] was also considered. This method does not use the arterial tracer concentration as input function.

As for the ROI approach, if the Logan graphical analysis is applied to a reference region ($Ref$), its equation becomes:

$$\frac{\int_0^t Ref(\tau)d\tau}{Ref(t)} = m\frac{\int_0^t C_p(\tau)d\tau}{Ref(t)} + q \quad (5.24)$$

where $C_p(t)$ is the plasma time activity curve, and $m$ and $q$ are parameters that become linear for time $t > t_*$, where $t_*$ correspond to the time when tracer in tissue is in equilibrium with plasma. Solving for $\int_0^t C_p(\tau)d\tau$ and rearranging parameters, the time activity of the radioligand in each pixel can be described by (MRTM$_0$, Ichise et al. 1996 [37]):

$$\frac{\int_0^t C_i(\tau)d\tau}{C_i(t)} = \frac{V_i}{V_{iRef}} \frac{\int_0^t Ref(\tau)d\tau}{C_i(t)} + \frac{V_i}{V_{iRef}k_{2Ref}} \frac{Ref(t)}{C_i(t)} + b \quad (5.25)$$
where $C_i(t)$ is the pixel time radioactivity, $V_i$ and $V_{iRef}$ are the total volumes of distributions of the pixel and reference region respectively; $k_{2Ref}$ is the clearance rate constant from the reference region to plasma, and $b$ is the intercept term, which becomes constant for $t > t^*$. Equation 5.25 allows for estimation of three parameters:

$$
\beta_1 = \frac{V_i}{V_{iRef}} \quad \beta_2 = \frac{V_{iRef}}{V_i} \quad \beta_3 = b
$$

(5.26)

by multilinear regression analysis for $t > t^*$. Assuming that the nondisplaceable distribution volumes in the tissue and reference regions are identical, the binding potential $BP$ is calculated from the first regression coefficient as:

$$
BP = \frac{V_i - V_{iRef}}{V_{iRef}} = (\beta_1 - 1)
$$

(5.27)

For radioligands with 1-TM, equation 5.25 is linear from $t = 0$ and $b$ is equal to $-1/k_2$ where $k_2$ is the clearance rate constant from the tissue to plasma. For 1-TM, $V_i = K_1/k_2$ and $V_{Ref} = K_{1Ref}/k_{2Ref}$, where $K_1$ and $K_{1Ref}$ are the rate constants for transfer from plasma to the tissue and the reference region, respectively, and, $R_1 = K_1/K_{1Ref}$, the relative radioligand delivery, can be calculated from the ratio of the second and third coefficients as $R_1 = -\beta_2/\beta_3$.

Ordinary LLS parameter estimation assumes that independent variables are noise-free. However, equation 5.25 contains noisy terms, $C_i(t)$, on the right-hand side (independent variables). In addition, the structure of equation 5.25 makes noise in the dependent (left-hand side) and independent variables correlated. These factors produce biased estimates [6, 39, 42]. Equation 5.25 can be rearranged to yield the following multilinear reference tissue model (MRTM1):

$$
C_i(t) = -\frac{V_i}{V_{iRef}b} \int_0^t R_{Ref} \tau d\tau + \frac{1}{b} \int_0^t C_i(\tau) d\tau - \frac{V_i}{V_{iRef}k_{2Ref}b} R_{Ref}(t)
$$

(5.28)
In equation 5.28, noisy \( C_i(t) \) is no longer present in the independent variables, although integral of \( C_i(t) \) is present. However, integrals of noisy data typically have much lower percent variation than the data themselves [39]. Additionally, the correlation of the noise in the dependent and independent variable is dramatically reduced in equation 5.28 compared with equation 5.25. Equation 5.28 also allows estimation of the three parameters:

\[
\begin{align*}
  \gamma_1 &= -\frac{V_t}{V_{i,Ref}b} \\
  \gamma_2 &= \frac{1}{b} \\
  \gamma_3 &= -\frac{V_t}{V_{i,Ref}k_{2,Ref}b}
\end{align*}
\]  

(5.29)

assuming that the integrals of \( C_i(\tau) \) and \( Ref(\tau) \), as well as \( Ref(t) \), are noise-free. \( BP \) can then be estimated by equation 5.28 from the ratio of the two regression coefficients as \( BP = -(\gamma_1/\gamma_2 + 1) \). If the pixel kinetics is described by a One-tissue compartment model, \( R_1 = \gamma_3 \) and \( k_2 = -\gamma_2 \).

Equation 5.28 also allows estimation of \( k_{2,Ref} \), which is given by \( k_{2,Ref} = \gamma_1/\gamma_3 \). However, a different value of \( k_{2,Ref} \) is estimated by 5.28 for each pixel, although there is only one reference region and therefore only one true value for \( k_{2,Ref} \). By fixing \( k_{2,Ref} \) to a value obtained with preliminary analysis as explained in [38] using 5.28, a two-parameter version of equation 5.28 is obtained by rearrangement to yield (MRTM2):

\[
C_i(t) = -\frac{V_t}{V_{i,Ref}b} \left( \int_0^t Ref(\tau)d\tau + \frac{1}{k_{2,Ref}} Ref(t) \right) + \frac{1}{b} \int_0^t C_i(\tau)d\tau \quad (5.30)
\]

Equation 5.30 can also be obtained by rearrangement of the graphical reference tissue model described by Logan et al. (1996, [28]). Equation 5.30 estimates two parameters, \( \gamma_1 = -V_t/V_{i,Ref}b \) and \( \gamma_2 = 1/b \) for \( t > t^* \), assuming that the integrals \( C_i(\tau) \) and \( Ref(\tau) \), as well as \( Ref(t) \), are noise-free. \( BP \) is then calculated from the ratio of the two regression coefficients as \( BP = -(\gamma_1/\gamma_2 + 1) \). For the 1-TM, \( R_1 = \gamma_1/k_{2,Ref} \) and \( k_2 = -\gamma_2 \). Because MRTM2 estimates fewer parameters, it is expected to be more stable than MRTM1, particularly at high-noise levels of pixel parametric imaging. This strategy has been shown to be effective in reducing noise from the three-
5. Pixel By Pixel Modeling

parameter simplified reference tissue model (SRTM) to the two-parameter model SRTM₂[71].

5.1.4 The Basis Function Method

SRTM can be also used at pixel level to generate parametric images of ligand binding. Since the application of this method at pixel level using a conventional nonlinear least squares approach to parameter estimation is slow and sensitive to noise, in this study SRTM has been applied as indicated by Gunn et al. in [59]. Briefly, the simplified reference tissue model can be rewritten as:

\[ C_i(t) = \theta_1 C_{\text{Ref}}(t) + \theta_2 C_{\text{Ref}}(t) \otimes e^{-\theta_3 t} \quad (5.31) \]

where \( \theta_1 = R_1 \), \( \theta_2 = k_2 - R_1 k_2 / (1 + BP) \) and \( \theta_3 = k_2 / (1 + BP) \). This new equation is linear in \( \theta_1 \) and \( \theta_2 \). Therefore for fixed values of \( \theta_3 \), \( \theta_1 \) and \( \theta_2 \) can be estimated using standard linear least squares. The nonlinear term can be dealt with by choosing a discrete spectrum of parameter values for \( \theta_3 \) and forming the corresponding basis functions:

\[ B_i(t) = C_{\text{Ref}}(t) \otimes e^{-\theta_3 t} \quad (5.32) \]

Equation 5.31 can then be transformed into a linear equation for each basis function:

\[ C_i(t) = \theta_1 C_{\text{Ref}}(t) + \theta_2 B_i(t) \quad (5.33) \]

This equation is then solved using linear least squares for each basis function. The index \( i \) for which the residual sum of squares is minimized is determined by a direct search and the associated parameter values for this solution are obtained \( (\theta_1, \theta_2, \theta_3) \). Values for \( BP \), \( R_1 \) and \( k_2 \) are then easily deduced from the relationships given in equation 5.31.

A logarithmic range of values for \( \theta_3 \) can be selected to encompass all plausible values for this parameter as governed by \( k_2 \) and \( BP \). This allows
the introduction of parameter bounds on $\theta_3$:

$$\theta_3^\text{min} \leq \theta_3 \leq \theta_3^\text{max}$$

$i = 1, \ldots, 303$ and where $\theta_3^\text{min} = k_2^\text{min}/(1 + B P^\text{max})$ and $\theta_3^\text{max} = k_2^\text{max}$. For ligand $[^{11}C]$-DASB considered in this study 303 discrete values for $\theta_3$ were found to be sufficient with $k_2^\text{min} = 0.0002 \text{ min}^{-1}$ and $k_2^\text{max} = 0.029 \text{ min}^{-1}$.

### 5.2 $[^{11}C]$-DASB Methods Results

In this study, the goal, with $[^{11}C]$-DASB, was to compare the performance of the presented methods in order to select the one that allows better \textit{in vivo} visualization of SERT at pixel level and to quantify the extent of possible biases introduced by the different approaches. The analysis was performed with MATLAB [21].

#### 5.2.1 Pixel Model Selection

The first step in the analysis is the selection of the compartmental model to be used for modeling $[^{11}C]$-DASB kinetics at pixel level. The model is usually a simplification of the one selected at ROI level because the pixel kinetics has lower signal-to-noise ratio than the ROI kinetics and is, therefore, more difficult to identify. The analysis performed at ROI level (see chapter 4), though, showed that $[^{11}C]$-DASB kinetics can be accurately described by 1-TM. This model is sufficiently simple and, therefore, is used to characterize also tracer kinetics at pixel level. Intravascular radioactivity $V_b$ is not included because its contribution at pixel level would be minimal (even if $V_b$ was, for example, 5%, it would be a negligible quantity in a single pixel) owing to the high $K_1$ values found at ROI level that indicate that $[^{11}C]$-DASB is highly extracted in all brain regions.
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5.2.2 Real Data Studies

With $[^{11}C]$-DASB the analysis was performed both on real and synthetic data. For what concerns the analysis of real data, the same 6 elderly healthy control subjects that were studied at ROI level were considered and methods Logan, GLLS, GWLLS and SRRSC were applied. All pixel identification methods except the Logan allow the generation of parametric images of rate constants $K_1$ and $k_2$. The parametric image of distribution volume $V_t$ can be derived as the ratio $K_1/k_2$ and the binding potential parametric image can also be calculated as:

$$BP = \frac{V_{tpixel} - V_{tCER}}{V_{tCER}}$$

(5.34)

Logan, instead, directly estimates $V_t$ and $BP$ can be calculated as in equation 5.34. In order to test the significance of difference between parametric images generated by the different methods, the Wilcoxon test was employed and $p<0.05$ was considered significant. Masks of ROIs CER, LOF, AMY, HIP and DRN were applied to the generated parametric images and for each region of interest a mean value and Standard Deviation (SD) were obtained. Mean values where calculated excluding parameter estimates having $BP > 50$ (for Logan, GLLS and GWLLS) and $CV > 100$ (for GWLLS, SRRSC and GRRSC).

5.2.3 Simulation Studies

For what concerns the simulation analysis Logan, GLLS, GWLLS, SRRSC, GRRSC, SRTM, MRTM$_1$ and MRTM$_2$ were considered. The analysis was performed on three planes of brain time activity which respectively included regions Mesial temporal cortex (MTC = AMY + HIP), Raphe (RAP) and Cerebellum (CER). Note that Logan graphical method was tested because it only requires Linear Least Squares (LLS) fitting and is an extensively used parametric imaging technique, even though it does not provide information on influx and efflux rate constants $K_1$ and $k_2$ nor on precision of $BP$ and $V_t$.

The plasmatic curve $C_p(t)$ of one of the elderly healthy control subject
5. Pixel By Pixel Modeling

(reference subject) preliminary studied on an ROI basis was considered as the input function. Parametric images of rate constants $K_1$ and $k_2$ and the parametric image of the a posteriori $\sigma$ estimated with WNLLS for the same subject were also considered. Note that $\sigma$ is a scalar value that reflects the noisiness of each pixel TAC. In fact, $\sigma$ (under the assumption that the covariance matrix of the measurement is expressed as in equation 5.16) is associated to the sum of the weighted residual evaluated at the point of convergence (WRSS), through the relation:

$$\sigma^2 = \frac{WRSS}{n_{\text{data}} - n_{\text{parameters}}}$$

therefore, for each pixel, the greater is WRSS the greater will be $\sigma$. Given $[K_1, k_2]^T$ and $C_p(t)$, the synthetic TAC for each pixel was generated according to 1-TM . True planes of brain activity were obtained. Gaussian noise was, then, added to the noise free planes. The Gaussian noise was assumed to have zero mean and a Standard deviation $SD$ given by:

$$SD_i = \alpha \left[ \sigma \sqrt{\frac{TAC(t_i)}{\Delta t_i}} \right]$$

$i = 1, ..., n$ where $TAC(t_i)$ is the pixel time activity value at time $t_i$, $\Delta t_i$ is the corresponding scan time interval, $\sigma$ is derived by WNLLS for a given pixel, $\alpha$ is a proportional constant that was set equal to 0 (no noise), 0.5, 1.25 (typical noise level found in vivo) and 1.5. 100 realization of noise for each value of $\alpha$ were considered to evaluate the statistical properties of the estimates of the parametric images in each pixel [9]. The noisy PET planes were identified using both arterial (Logan, GLLS, GWLLS, GRRSC and SRRSC) and non arterial methods (MRTM$_1$, MRTM$_2$ and SRTM) and, for each algorithm and for each $\alpha$, a set of parametric images ($K_1$, $k_2$, $V_t$ and $BP$ for arterial methods and $BP$, $R_1$ and $k_2$ for non arterial methods) were obtained. When possible (GWLLS, GRRSC, SRRSC, (MRTM$_1$, MRTM$_2$ and SRTM)), parametric images of parameter precisions (CVs) were derived. Masks of the ROIs included in the simulated planes were applied to the
parametric images and for each region of interest a mean value and Standard Deviation (SD) were obtained.

For arterial methods estimated CV values were considered, when possible, in order to exclude outlier pixels (CV >100% meant failure in the fitting process). For non arterial methods and GLLS, instead, parameter estimates were considered outliers if their values were less than zero or more than five times the true values (as in Ichise et al., 1996, [37]). Bias of parameters and the Root Mean Squared Error (RMSE) was calculated as:

\[
\text{Bias}\% = \frac{1}{N} \sum_{i=1}^{N} \left( \frac{p_i - p}{p} \right) \times 100
\]

\[
\text{RMSE}\% = \frac{1}{(N-1)} \sqrt{\sum_{i=1}^{N} \left( \frac{p_i - p}{p} \right)^2} \times 100
\]

where \( p_i \) is the estimated parameter, \( p \) is the true value and \( N \) is the number of repeated realizations (i.e., 100 in this case). Parametric images of Bias and RMSE were generated for each implemented method.

5.2.4 Imaging Results

The analysis, as stated above, was performed on the 6 elderly healthy control subjects that were studied at ROI level. Methods GLLS, GWLLS, SRRSC and Logan were applied.

Results reported in this study will focus on Logan and GWLLS performance, since Logan method showed, due to the high noise level of pixel TACs, to underestimate \(^{11}\text{C}\)-DASB binding in high SERT density regions while GWLLS gave very similar results to SRRSC and GRRSC but it required a shorter time to generate parametric images of binding. GLLS, moreover, performed worse than GWLLS, SRRSC and GRRSC and it also had the limit that it did not provide information on parameter precision (which proved to be of tremendous help in the understanding of the fitting process).

Mean BP values are shown in Table 5.1. The analysis showed that GWLLS method gives higher BP values than LOGAN mainly in DRN, for which quantification is most challenging given its low signal to noise ratio. For instance, in one representative subject \( BP_{LOGAN} \) in DRN was 3.47 ± 0.77
Table 5.1: BP(mean±SD) values. SD is the standard deviation between subjects (* = calculated over 4 subjects, ‘ = calculated over 3 subjects)

<table>
<thead>
<tr>
<th></th>
<th>LOGAN mean ± SD</th>
<th>GWLLS mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CER</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>LOF</td>
<td>0.19 ± 0.08</td>
<td>0.16 ± 0.07</td>
</tr>
<tr>
<td>HIP</td>
<td>0.41 ± 0.17</td>
<td>0.44 ± 0.21</td>
</tr>
<tr>
<td>AMY *</td>
<td>0.81 ± 0.22</td>
<td>1.04 ± 0.16</td>
</tr>
<tr>
<td>DRN ’</td>
<td>2.68 ± 1.21</td>
<td>5.72 ± 1.36</td>
</tr>
</tbody>
</table>

(mean ± SD), while $BP_{GWLLS}$ was $4.15 ± 0.79$ (in this case SD represents the pixel variability within the ROI). Wilcoxon test indicated that the difference between GWLLS and LOGAN BP was statistically significant for all six subjects in all planes including brain structure important for $[^{11}C]$-DASB.

Another important results, often found in neuroreceptor systems, is that the difference between methods varies across regions (see Table 5.1). It is higher in 'high to medium' receptor density regions (DRN, AMY and HIP) and it reduces in low receptor density regions (LOF and CER). Equivalent results can be derived by considering the associated parametric images.

Fig 5.1 shows typical $BP$ images obtained with GWLLS (left column) and Logan (middle column) in two planes of the brain including regions at low and high receptor density. In particular plane I includes portions of HIP, AMY and DRN, plane II includes a portion of DRN. GWLLS gives higher $BP$ values, mainly in DRN, however, difference between GWLLS and Logan decreases for ROIs associated to low receptor density (Fig. 5.1 right column).

Unlike Logan, GWLLS, SRRSC and GRRSC also provided influx and efflux rate constants $K_1$ and $k_2$ (Fig.5.2 ) as well as precision of all parameters (images not shown). For what concerns the parameter precision, all three methods gave mean CV of parameter estimates mainly in the range of 10 to 50% for all subjects in all ROIs. Note, though, that mean parameter and precision values were calculated, for each ROI, including only pixels whose CVs were <100%. The presence, in fact, of even just one pixel in the ROI where the fitting process failed can corrupt the entire estimate and bring totally unreliable final mean values if CVs are not considered.
5. Pixel By Pixel Modeling

Figure 5.1: $BP$ parametric images and difference between GWLLS and LOGAN methods

Figure 5.2: $K_1$ and $k_2$ parametric images given by the GWLLS method
In conclusion, the application of Logan, GLLS, GWLLS, SRRSC and GRRSC methods to real subjects data at a pixel by pixel level showed, that GWLLS, SRRSC and GRRSC can generate parametric $BP$ images of greater dynamic range than those generated by Logan and that these images are in more agreement with the known physiological distribution of SERT receptors. Moreover, $BP$ differences between GWLLS and Logan are greater in regions that are important for SERT binding such as DRN, AMY, etc. GWLLS method appears, therefore, to be a good alternative to improve parametric $[^{11}C]$-DASB quantification because it can be readily applied on a voxel basis and provides information concerning both radiotracer transport and parameter precision. The accuracy of GWLLS and all other tested methods was, anyway, quantified by simulation as explained in the next section.

5.2.5 Simulation Results

Matlab simulation analysis was performed as previously explained and parametric images were generated using methods Logan, GLLS, GWLLS, SRRSC, GRRSC, SRTM, MRTM$_1$ and MRTM$_2$. Only results relative to $\alpha = 0$ (no noise added) and $\alpha = 1.25$ (typical noise level found in vivo) will be reported for space problems.

Let’s first focus on the performance of compartmental methods with arterial input function. When $\alpha = 0$ (no noise added) all arterial methods generated parametric images of very good quality, associated to almost zero mean regional Bias and RMSE. The mean Bias was always between 0 and 1% for all methods in CER, HIP and AMY. It was between 0 and 2% in the DRN region.

Results were different when the case $\alpha = 1.25$ was considered. Below are typical parametric images of the true binding potential ($BP$) (Fig. 5.3) and true tissue distribution volume ($V_t$) (Fig. 5.4) with their relative RMSEs in a plane of time activity including the Raphe region. Images of the true $BP$ and $V_t$ for that plane are in the upper left corner of both figures. The other images represent the RMSEs calculated as explained in equation 5.37 for all the arterial methods. Note that the scale of the RMSE images has
Figure 5.3: BP parametric images
Figure 5.4: \( V \) parametric images
been compressed so that RMSE higher than 50% would appear more visible. As it can be seen by visual inspection of figures 5.3 and 5.4, when $\alpha = 1.25$ (presence of a level of noise typically found in vivo), the most biased method is Logan, followed by GLLS. Instead, the weighted version of GLLS (GWLLS) and the Ridge Regression algorithms (SRRSC and GRRSC) perform better. Their RMSE images are all quite similar throughout the plane.

These remarks become more evident if Table 5.2 is considered. Table 5.2 contains mean values and Standard Deviation (SD) of parameters calculated by applying the ROI masks on the obtained parametric images.

From table 5.2 it is evident that parameters $K_1$ and $k_2$ are well estimated by all methods in all regions. $K_1$ bias is between 0 and -1% in all regions, while $k_2$ bias is between -2 and 1% in CER, between -2 and -3% in AMY, between -3 and 0 in HIP and between 0 and 2% in DRN.

When the typical noise level found in vivo is considered ($\alpha = 1.25$), $V_t$ and $BP$ estimates are biased. Remember that for Logan and GLLS methods, pixel associated to CV values less than 100% and estimated parameter greater than 5 times their true values were considered as outliers. With GWLLS, SRRSC and GRRSC methods, only the CV constraint was considered. The $BP$ and $V_t$ bias varies with respect to the region of interest and the algorithm used. What is very evident is BP Logan underestimation, which varies from $\sim$0% in the CER region and reaches 69% in the DRN region. The Logan $V_t$ underestimation is 3 and 56% in CER and DRN respectively. This result is confirmatory of what had already been noticed in vivo [64] and presented in section 5.2.4. The GLLS $BP$ and $V_t$ mean ROI values are also biased. The bias is less evident, though, than with Logan. $BP$ bias is 0% in CER and reaches 31% in DRN, while $V_t$ Bias is 2% in CER and 26% DRN.

Methods GWLLS, SRRSC and GRRSC perform definitely better. The $BP$ GWLLS and GRRSC Bias is between 0% and 10% in all region, while it is between 0% and 11% with SRRSC. The $V_t$ bias is between 0% and 3% in all regions for GWLLS and GRRSC and between -1 and 7% for SRRSC. The bar diagrams in Fig. 5.5 are ROI-based representations of the information included in figures 5.3, 5.4 and Table 5.2 ($BP$ in CER has not been considered in Fig. 5.5 upper graph, since it is equal to zero.). Still the Lo-
<table>
<thead>
<tr>
<th>Parameter</th>
<th>ROI</th>
<th>TRUE</th>
<th>LOGAN</th>
<th>GLLS</th>
<th>GWLLS</th>
<th>SRRSC</th>
<th>GRRSC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>sd</td>
<td>mean</td>
<td>sd</td>
<td>mean</td>
<td>sd</td>
<td>mean</td>
</tr>
<tr>
<td>BP CER</td>
<td>0.000</td>
<td>0.096</td>
<td>0.000</td>
<td>0.105</td>
<td>0.000</td>
<td>0.113</td>
<td>0.000</td>
</tr>
<tr>
<td>AMY</td>
<td>0.417</td>
<td>0.361</td>
<td>0.379</td>
<td>0.332</td>
<td>0.436</td>
<td>0.401</td>
<td>0.380</td>
</tr>
<tr>
<td>HIP</td>
<td>1.706</td>
<td>1.455</td>
<td>1.010</td>
<td>0.595</td>
<td>1.984</td>
<td>3.011</td>
<td>1.756</td>
</tr>
<tr>
<td>DRN</td>
<td>3.581</td>
<td>2.682</td>
<td>1.100</td>
<td>0.853</td>
<td>4.709</td>
<td>5.882</td>
<td>3.656</td>
</tr>
</tbody>
</table>

| HIP       | 45.520 | 24.478 | 32.723 | 9.687 | 50.949 | 51.412 | 46.453 | 28.149 |
| DRN       | 77.054 | 45.114 | 34.186 | 13.886 | 97.463 | 100.422 | 82.559 | 61.816 |

| K1 CER    | 0.417 | 0.047 | 0.411 | 0.054 | 0.416 | 0.053 | 0.418 | 0.043 |
| AMY       | 0.411 | 0.071 | 0.406 | 0.074 | 0.411 | 0.073 | 0.406 | 0.065 |
| HIP       | 0.377 | 0.050 | 0.375 | 0.053 | 0.378 | 0.052 | 0.373 | 0.045 |
| DRN       | 0.358 | 0.033 | 0.354 | 0.039 | 0.358 | 0.039 | 0.357 | 0.027 |

| k2 CER    | 0.025 | 0.004 | 0.025 | 0.005 | 0.025 | 0.005 | 0.025 | 0.004 |
| AMY       | 0.018 | 0.004 | 0.018 | 0.005 | 0.018 | 0.004 | 0.017 | 0.004 |
| HIP       | 0.010 | 0.004 | 0.010 | 0.004 | 0.010 | 0.004 | 0.010 | 0.004 |
| DRN       | 0.007 | 0.005 | 0.007 | 0.005 | 0.007 | 0.005 | 0.007 | 0.005 |

Table 5.2: mean ±SD regional values of parameters by different estimation approaches
gan underestimation is very evident, and it is also evident the fact that this underestimation varies across regions. GLLS method, instead, appears to overestimate $BP$ and $V_t$ especially in high receptor density regions (AMY and DRN) and the pixel variability around the mean value (SD) is greater than other methods. GWLLS, SRRSC and GRRSC give almost identical results except for a small overestimation of SRRSC in AMY and DRN. It is clear, then, that these bar diagrams show that the two arterial methods that generate less biased parametric images in all regions are GWLLS and GRRSC. The selection of the best arterial method for [11C]-DASB parametric imaging between these two has to be, then, based on other issues like the computational time.

Results relative to the application of compartmental methods without arterial input function were only retrieved for regions AMY and DRN. Being SRTM, MRTM$_1$ and MRTM$_2$ reference tissue methods, they cannot be applied, by definitions, on CER. Moreover, based on the results from the arterial methods, we decided to focus on medium-to-high receptor density regions of small (DRN) and medium (AMY) size. Notice, also, that many
different approaches were considered for the estimation of $k_{2\text{Ref}}$ in MRTM$_2$. $k_{2\text{Ref}}$ was estimated as indicated in Ichise et al. [38] as a weighted mean value of different ROIs, and different types of weighting were also considered. Anyway, MRTM$_2$ results reported below are relative to the hypothesis that $k_{2\text{Ref}}$ is perfectly estimated. The perfect $k_{2\text{Ref}}$ that was used in this study was the mean regional true $k_2$ value of CER. Table 5.3 report mean regional $BP$, $R_1$ and $k_2$ values and their associated SD in regions AMY and DRN calculated on parametric images obtained with methods SRTM, MRTM$_1$ and MRTM$_2$. Taking a look at table 5.3 it is possible to notice that, unlike the arterial methods, estimates of parameters are biased when $\alpha = 0$. $R_1$ is the more stable parameter. When $\alpha = 0$, in fact, its bias is 0% for methods MRTM$_1$ and MRTM$_2$, and is only -2% in AMY and -1% in DRN for method SRTM. $BP$ Bias is between -6% (MRTM$_1$) and 13% (SRTM) in AMY and between 3 and 5% in DRN for MRTM$_1$ and SRTM respectively. The more unstable parameter is, instead, $k_2$. Its bias in AMY is only 1% for MRTM$_2$, 10% for MRTM$_1$ but reaches -30% for SRTM. In DRN $k_2$ bias varies: it is 0% for MRTM$_2$, it is 9% for MRTM$_1$ and becomes 76% for SRTM. Things are even worse when a typical noise level found in vivo is considered ($\alpha = 1.25$). Again, the more stable parameter is $R_1$ even if its bias becomes more evident when $\alpha = 1.25$. Still MRTM$_2$ method introduces a very low bias in both regions (only 1%). This bias gets higher for MRTM$_1$ (-6% in AMY and -9% in DRN) and for SRTM becomes very evident (-18% an AMY and -20% in DRN). Analogous patterns are found for $BP$ and $k_2$ bias. $BP$ Bias becomes -38 and -55% in AMY and DRN for MRTM$_1$ while it is between -17 and 6% for the other methods. MRTM$_2$ $k_2$ bias is 1% in AMY and 2% in DRN, it becomes 17% in AMY and 166% in DRN for SRTM and is over 100% in both regions for MRTM$_1$. Part of the results in Table 5.3 is represented in the bar diagrams in Fig. 5.6. Results, in conclusion, indicate that non arterial methods are biased even when $\alpha = 0$. In particular, method MRTM$_2$ would potentially produce good estimates of parameters if the correct value for $k_{2\text{Ref}}$ could be selected.
<table>
<thead>
<tr>
<th></th>
<th>DRN</th>
<th>AMY</th>
<th>DRN</th>
<th>AMY</th>
</tr>
</thead>
<tbody>
<tr>
<td>α = 0</td>
<td>0.905 ± 0.005</td>
<td>0.905 ± 0.007</td>
<td>0.858 ± 0.005</td>
<td>0.858 ± 0.005</td>
</tr>
<tr>
<td>α = 1.25</td>
<td>0.905 ± 0.007</td>
<td>0.905 ± 0.012</td>
<td>0.858 ± 0.009</td>
<td>0.858 ± 0.009</td>
</tr>
</tbody>
</table>

Table 5.3: Mean regional BP, \( R_1 \) and \( k_2 \) values for non arterial methods.
5.2.6 Discussion

The analysis was performed both on real and synthetic data. Results indicate that GWLLS, SRRSC and GRRSC can generate parametric BP images of greater dynamic range than those generated by Logan. These images are more in agreement with known distribution of SERT receptors. BP differences between GWLLS and Logan were statistically significant and greater in regions that are important for SERT binding such as DRN, AMY, etc. Therefore, GWLLS method is the best for parametric \([^{11}C]\)-DASB quantification because it provides accurate and precise information concerning both radiotracer transport and parameter precision in a reasonably short amount of time.

The accuracy of all methods was quantified by simulation. Results confirmed that, between the arterial methods (Logan, GLLS, GWLLS, SRRSC and GRRSC), GWLLS and GRRSC are the ones that generate less biased parametric images in all regions in presence of a noise level typically found \textit{in vivo} (\(\alpha = 1.25\)), while in absence of noise (\(\alpha = 0\)) all methods give good estimates and good parameter precisions. Non arterial methods (SRTM by Gunn, MRTM\(_1\) and MRTM\(_2\) ) were only quantified for AMY and DRN regions and they gave, unlike the arterial methods, biased estimates of parameters even when \(\alpha = 0\). Results were even worse when \(\alpha = 1.25\) was considered. The bias was very high for \(k_2\), in particular for methods SRTM

\[\text{Figure 5.6: regional BP and } k_2 \text{ (mean } \pm \text{SD) histograms}\]
5. Pixel By Pixel Modeling

and MRTM₁, while R₁ resulted the most stable parameter at all noise levels.

Method MRTM₂ needs to be discussed with more attention, since it introduced a very little bias for all parameters at all noise levels when \( k_{2\text{Ref}} \) was a priori set equal to the true value for CER. This method introduced by Ichise et al. in 2003 clearly seems to improve parameter estimation at pixel level and would, therefore, allow rapid generation of stable parametric images of binding and relative delivery for \([11C]-DASB \) PET. Anyway, this method requires accurate a priori estimation of parameter \( k_{2\text{Ref}} \), which was not easily determined in this study. In the paper by Ichise et al. [38], \( k_{2\text{Ref}} \) is derived as a weighted average of several \( k_{2\text{Ref}} \) samples form high-BP ROIs, previously analyzed by MRTM₁. Recently, a test-retest study was published by Kim and his collaborators [29] where the steps for \( k_{2\text{Ref}} \) calculation on real data were defined. \( k_{2\text{Ref}} \) was calculated in two steps: first, preliminary \( R₁ \) images were generated with a \( k_{2\text{Ref}} \) value estimated by MRTM₁. Then, the subject’s MR images were coregistered to the preliminary \( R₁ \) images in SPM2 and fused using PMOD. Several anatomic ROIs were manually defined on these fused images and the ROIs were placed on the motion-corrected dynamic data to obtain ROI TACs from Raphe, Striatum and Thalamus. Then MRTM₁ was applied on these regional TACs and estimated \( k_{2\text{Ref}} \) values are averaged (weighted average) to obtain the final \( k_{2\text{Ref}} \) to be used with MRTM₂. This is a complicated procedure for routine analysis, also dependent on the weights chosen for the average calculation. Different weightings for \( k_{2\text{Ref}} \) were considered in this study but it was not possible to define a systematic way to retrieve the correct \( k_{2\text{Ref}} \) value and data analysis (results not shown), unlike Kim et al. [29], indicated that even when slightly different from the true \( k_{2\text{Ref}} \) were chosen, method MRTM₂ suddenly produced very biased estimates. For these reasons, in absence of an arterial curve, the SRTM should be applied on real data for BP parametric images generation, considering that in high receptor density regions the bias varies between -17 and 13%. Results also indicate that with SRTM, \( k₂ \) parametric images can be produced but they are not very accurate.

Finally, the scanning time duration needs to be considered. Data acquired in this study are collected over a 90 mins experiment, while recently
publications on $[^{11}C]$-DASB are all relative to acquisitions made over 120 min [29, 60, 68]. The scanning time duration is a major issue, since it may appear logical than the longer the acquisition time, the more accurate the estimation. However, because of the physiological decay of $^{11}C$, as well as subject head motion, the acquired data becomes increasingly noisy towards the end of the scan and does not necessarily contribute useful information.
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Chapter 6

Conclusions

Positron emitter tracers [carbonyl $^{11}$C]WAY-100635 and $^{11}$C-DASB allow the in vivo study of serotonin receptors 5-HT$_{1A}$ and SERT, respectively, and permit the quantification of several important physiological parameters like the tracer distribution volume ($V_t$) and the binding potential ($BP$).

PET quantification of the serotonin system can be performed at two different levels, ROI and pixel levels, which are complimentary. In this study, some of the recent findings and issues relative to modeling and identification methods of these two tracers are discussed.

6.1 $^{11}$C-DASB Quantification

The goal of $^{11}$C-DASB study was to determine the best compartmental model to be used for ROI quantification and, at pixel level, to select the best identification method for fast and accurate generation of parametric images of ligand binding. To do so, six subjects full PET dynamics kindly made available by Dr. Julie Price of the PET Facility of the Pittsburgh Medical Center (Pittsburgh, PA, USA) were studied.

At ROI level, Spectral analysis indicated a single tissue compartment to model the regional time activity curve. Six different compartmental models with arterial input function based on either One- or Two-tissue compartments and involving two to four rate constants were identified by WNLLS and the
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preferred method was 1-TM with \( V_b=5\% \) (method B). Methods based on Two-Tissue compartments failed to converge in all subjects. The Reference Tissue methods (standard RTM and simplified SRTM) were also applied using the Cerebellum as reference region. Both these methods gave biased results with respect to method B, in particular in high receptor density regions as AMY and DRN, and SRTM performed better than RTM. This latter result was expected since RTM assumes a region of interest to be modeled by a Two-Tissue compartment model while it was found that the best arterial compartmental model for \([^{11}C]-DASB\) quantification is a One-Tissue compartment model. Logan graphical analysis was also applied since it is a very frequently used method and it was found that at ROI level this method gives BP and \( V_t \) estimates in agreement to method B. Logan BP, in fact, only slightly underestimated with respect to method B, with underestimation (mean values over subjects) between 1\% and 7\% in all ROIs except in AMY where it was 18\%. Based on the results briefly indicated here and extensively presented in Chapter 4, model 1-TM with \( V_b=5\% \) was selected as the compartmental model of choice for \([^{11}C]-DASB\) quantification at ROI level.

For what concerns the parametric imaging problem, the arterial compartmental model selected for quantification at ROI level was also employed for modeling \([^{11}C]-DASB\) pixel time activity curves, even though pixel TACs are characterized by lower signal to noise ratio which prevent Non Linear Least Squares identification. Two popular parametric imaging methods are the conventional standard Logan graphical method, which only requires Linear Least Squares (LLS) fitting, and the Simplified Reference Tissue method (SRTM) which also requires only LLS fitting when implemented as in Gunn et al. (1997). The analysis was performed both on real and synthetic data. Pixel analysis on real data was performed on the same six elderly healthy control subjects that were studied at ROI level and methods Logan, GLLS, GWLLS and SRRSC were applied. Results showed, that GWLLS, SRRSC and GRRSC can generate parametric BP images of greater dynamic range than those generated by Logan and that these images are in more agree-
ment with the known physiological distribution of SERT receptors. Moreover, BP difference between GWLLS and Logan were greater in regions that are important for SERT binding such as DRN, AMY, etc. Thus, GWLLS method appears as a good alternative to improve parametric $[^{11}\text{C}]-$DASB quantification because it can be readily applied on a pixel basis and provides information concerning both radiotracer transport and parameter precision.

The accuracy of GWLLS and all other tested methods was, also, quantified by simulation. Simulation analysis was performed on three planes of brain activity at different noise levels and methods Logan, GLLS, GWLLS, SRRSC, GRRSC, SRTM, MRTM$_1$ and MRTM$_2$ were considered, and GWLLS was the identification method of choice for fast and accurate generation of ligand binding images.

A still open issue for $[^{11}\text{C}]-$DASB quantification is the scanning time duration. In fact, it may appear logical that the longer the acquisition time, the more accurate the estimation. However, because of the physiological decay of $^{11}\text{C}$, as well as subject head motion, the data become increasingly noisy towards the end of the scanning time. The data in this study where acquired over a 90 min experiment, while recently some studies were published with data acquired over 120 min. In the future, further efforts are necessary to understand if and how much a longer scanning time could improve parameter estimation of this ligand binding.

6.2 $[^{11}\text{C}]$WAY-100635 Quantification

The goal of $[^{11}\text{C}]$WAY-100635 study was to determine the best compartmental model to be used for ROI quantification and to study the validity domain of the frequently applied Reference Tissue Methods. To do so, nine subjects full PET dynamics kindly made available by Dr. Julie Price of the PET Facility of the Pittsburgh Medical Center (Pittsburgh, PA, USA) were studied.

Spectral Analysis indicated as three the number of tissue compartment to model all ROIs except HIP and DRN, where two tissue compartments were sufficient. The selection of the three-exponential model, anyway, although
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Theoretically possible was quite a novel result. Therefore, in order to validate this finding, the possibility that a different metabolite fraction model could affect Spectral Analysis results was explored. Analysis performed on four of the nine subjects indicated that the model used to model $[^\text{11C}]\text{W A Y-100635}$ metabolite fraction potentially has an impact on the number of the spectral components. Anyway, results obtained on the entire data set of nine subject confirmed the three-exponential model (i.e. a three tissue compartments) as the most parsimonious in all ROIs, except HIP and DRN, regardless of the metabolite fraction model.

Based on this result, four compartmental models with arterial input function were compared. Parameter estimation was successful for all models in almost all regions, and the preferred model was a Three-Tissue compartment model in all ROIs except HIP and DRN, where a Two-Tissue compartment model was selected as the most parsimonious.

The identification of the Three-Tissue compartment model allowed, also, to obtain a very important results about the physiological interpretation of the compartment kinetics of the standard Two-Tissue compartment model. In fact, by plotting $[^\text{11C}]\text{W A Y-100635}$ compartmental kinetics predicted by the Two- and Three-Tissue compartment models, it is evident that the shape of the compartmental predicted dynamics suggests a novel physiological interpretation of the compartmental kinetics in the Two-Tissue compartment model: the first tissue compartment represents mainly free kinetics (and not “free + non specific” as in the standard interpretation), and the second tissue compartment is a combination of specific and part of the nonspecific components (and not only specific binding).

Models RTM and SRTM applied on the real dataset gave quite disappointing results. Both RTM and SRTM produced heavy under and over estimation of parameters and, surprisingly, they gave equal BP values, as if the more information included in RTM didn’t help the identification process. This could be due to the fact that the hypotheses underlying the Reference Tissue models were not valid in the Cerebellum region. A Three-Tissue model was, in fact, resolvable in Cerebellum in six of the nine subjects with good precision and this suggested the presence of two non specific compartments.
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A simulation study was, then, performed in order to understand if and how much the violation of the Reference Tissue model assumptions of $[^{11}\text{C}]\text{WAY-100635}$ could affect the final results obtained by RTM and SRTM. To do so, the three hypothesis on which these methods are based were tested: CER modeled with a One-Tissue compartment model, $V_b$ equal to 0%, and $V_{f+ns}(\text{Ref})$ (i.e. $V_{ICER}$) equal to $V_{f+ns}(\text{ROI})$. Simulations indicated that a correct estimation of the binding potential with RTM and SRTM models is possible only if all the assumption underlying these models are verified. As soon as one of them fails, BP and the other RTM and SRTM parameters are biased, confirming the results that are obtained in this study at ROI level. Simulation analysis also shows that only when the system under study has a model structure equal to that assumed by RTM, i.e. the CER region described by a single compartment and ROI modeled by a 2-TM, then $BP_{SRTM}$ differentiate from the $BP_{RTM}$. In all other cases where CER has a more complex structure than a One-Tissue compartment model, it was found $BP_{SRTM}$ always equal to $BP_{RTM}$.

The simulation analysis, in conclusion, proved that Reference Tissue models are biased because the hypothesis underneath these methods are not valid for $[^{11}\text{C}]\text{WAY-100635}$, and therefore their use for $[^{11}\text{C}]\text{WAY-100635}$ quantification should be avoided or, if the arterial curve is not available, the biases reported in this study should be kept in mind. When the arterial information is available, instead, the Three-Tissue compartment is the compartmental model of choice for $[^{11}\text{C}]\text{WAY-100635}$ quantification at ROI level in all regions except regions HIP and DRN. Regions HIP and DRN are better modeled by a Two-Tissue compartment model.

Finally, an open issue for $[^{11}\text{C}]\text{WAY-100635}$ is the possibility to generate parametric images of this ligand binding. This issue is difficult to solve because of many problems concerning both the modeling and the parameter identification methods. A first problem, for example, would be the selection of the model to be implemented at pixel level for $[^{11}\text{C}]\text{WAY-100635}$ kinetics. The Three-Tissue compartment model is, in fact, too complicated and should be further reduced to a sim-
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A second problem would be the low signal to noise ratio of $[^{11}\text{C}]\text{WAY-100635}$ that makes the signal difficult to identify even with a simpler model, and even though a first research direction could be the application to $[^{11}\text{C}]\text{WAY-100635}$ of the methods presented in this study, that proved to be successful for $[^{11}\text{C}]-\text{DASB}$, it is not guaranteed that they will be for $[^{11}\text{C}]\text{WAY-100635}$. 
APPENDIX A

The Three tissue Compartmental model is well known from literature. It is represented as:

Its equation are given by the mass balance in each compartment:

\[
\begin{align*}
\frac{dC_f(t)}{dt} &= K_1 C_p(t) - (k_2 + k_3 + k_4)C_f(t) + k_4 C_s(t) + k_6 C_{ns}(t) \\
\frac{dC_s(t)}{dt} &= k_3 C_f(t) - k_4 C_s(t) \\
\frac{dC_{ns}(t)}{dt} &= k_5 C_f(t) - k_6 C_{ns}(t)
\end{align*}
\]

with initial conditions: \(C_f(0) = C_s(0) = C_{ns}(0) = 0\) where \(C_p(t)\) represents the amount of \([\text{carbonyl} \ ^{11}C]\text{WAY-100635}\) in plasma and \(C_f(t), C_s(t)\) and \(C_{ns}(t)\) is the ligand not bound to any receptor, bound to its target receptors \((5-\text{HT}_{1A})\) and bound to non specific receptors in the brain region of interest under study respectively. The total amount measured by PET is the sum of the amounts present in the vascular and intracellular space of the ROI. It is

![Diagram](image)

Figure 6.1: The Three-tissue compartment model.
6. Conclusions

given by:

\[ C_i(t) = (1 - V_b)(C_f(t) + C_s(t) + C_{ns}(t)) + V_b C_b(t) \]  \hspace{1cm} (6.2)

where \( V_b \) [ml ml\(^{-1}\)] is the vascular volume of the ROI and \( C_b(t) \) represents the whole tracer concentration.

We show here that the model defined by equations 6.2 and 6.2 is a priori non uniquely identifiable and that it admits two symmetric solutions for parameters \((k_4, k_6)\) and \((k_3, k_5)\). To solve the identifiability problem we use the transfer function method. Laplace transforms of model equations are:

\[
sC_f(s) = K_1 C_p(s) - (k_2 + k_3 + k_5)C_f(s) + k_4 C_s(s) + k_6 C_{ns}(s)
\]

\[
sC_s(s) = k_3 C_f(s) - k_4 C_s(s)
\]

\[
sC_{ns}(s) = k_5 C_f(s) - k_6 C_{ns}(s)
\]

with initial conditions: \( C_f(0) = C_s(0) = C_{ns}(0) = 0 \). Solving for concentrations in each compartment gives:

\[
C_f(s) = \frac{K_1 (s+k_4) (s+k_6)}{s^3 + s^2 (k_2 + k_3 + k_4 + k_5 + k_6) + s (k_2 k_3 + k_2 k_6 + k_4 k_6 + k_5 k_6) + k_2 k_3 k_6 C_p(s)}
\]

\[
C_s(s) = \frac{K_1 k_5 (s+k_4)}{s^3 + s^2 (k_2 + k_3 + k_4 + k_5 + k_6) + s (k_2 k_3 + k_2 k_6 + k_4 k_6 + k_5 k_6) + k_2 k_3 k_6 C_p(s)}
\]

\[
C_{ns}(s) = \frac{K_1 k_3 (s+k_3)}{s^3 + s^2 (k_2 + k_3 + k_4 + k_5 + k_6) + s (k_2 k_3 + k_2 k_6 + k_4 k_6 + k_5 k_6) + k_2 k_3 k_6 C_p(s)}
\]

thus Laplace transform of \( C_i(t) \) can be written as:

\[
C_i(s) = (1 - V_b) \left( \frac{K_1 s^2 + s K_1 (k_3 + k_4 + k_5 + k_6)}{s^3 + s (k_2 + k_3 + k_4 + k_5 + k_6) + s (k_2 k_3 + k_2 k_6 + k_4 k_6 + k_5 k_6) + k_2 k_3 k_6} + \right.
\]

\[
+ \frac{K_1 s^2 + s K_1 (k_3 + k_4 + k_5 + k_6)}{s^3 + s (k_2 + k_3 + k_4 + k_5 + k_6) + s (k_2 k_3 + k_2 k_6 + k_4 k_6 + k_5 k_6) + k_2 k_3 k_6} C_p(s) + V_b C_b(s)
\]  \hspace{1cm} (6.5)
6. Conclusions

The exhaustive summary of the model is given by:

\[ K_1 = \alpha \]
\[ K_1 (k_3 + k_4 + k_5 + k_6) = \beta \]
\[ K_1 (k_4 k_6 + k_3 k_6 + k_4 k_5) = \gamma \]
\[ (k_2 + k_3 + k_4 + k_5 + k_6) = \delta \]
\[ (k_2 k_4 + k_2 k_6 + k_4 k_6 + k_3 k_6 + k_4 k_5) = \epsilon \]
\[ k_2 k_4 k_6 = \eta \]

(6.6)

where \( \alpha, \beta, \gamma, \delta, \varepsilon \) and \( \eta \) are positive quantities that can be estimated from the data.

Since parameter \( K_1 \) and \( V_b \) are \textit{a priori} uniquely identifiable (see 6.6), the second addend of equation 6.5 is a known quantity; therefore, it is possible to concentrate only on the first term of the sum without considering the constant multiplication factor \( (1-V_b) \):

\[
\left[ \frac{K_1 s^2 + s K_1 (k_3 + k_4 + k_5 + k_6) + K_1 (k_4 k_6 + k_3 k_6 + k_4 k_5)}{s^3 + s^2 (k_2 + k_3 + k_4 + k_5 + k_6) + s (k_2 k_4 + k_2 k_6 + k_4 k_6 + k_3 k_6 + k_4 k_5) + k_2 k_4 k_6} \right]
\]

(6.7)

It is easy to show that, solving the system equation represented by the exhaustive summary, model parameters \( K_1, k_2, k_3, k_4, k_5 \) and \( k_6 \) can be expressed as combinations of the known parameters \( \alpha, \beta, \gamma, \delta, \varepsilon \) and \( \eta \).

From system equations 6.6 it is possible to write the model parameters as follows:

\[ K_1 = \alpha \]
\[ k_2 = \left[ \delta - \frac{\beta}{\alpha} \right] \]
\[ k_3 = \frac{\beta}{\alpha} - (k_4 + k_6) - k_5 \]
\[ k_5 = \frac{1}{(k_4 - k_6)} \left[ \frac{\gamma}{s} - (k_4 k_6) - k_6 (\frac{\beta}{\alpha} - (k_4 + k_6)) \right] \]
\[ (k_4 + k_6) = \frac{\epsilon - \gamma}{\delta - \frac{\beta}{\alpha}} \]
\[ k_4 k_6 = \frac{\eta}{\delta - \frac{\beta}{\alpha}} \]

(6.8)

Notice that, once you have resolved parameters \( k_4 \) and \( k_6 \) as functions of
6. Conclusions

\(\alpha, \beta, \gamma, \delta, \varepsilon\) and \(\eta\), it is possible to estimate all the other parameters of the model.

\(k_4\) and \(k_6\) can be calculated resolving the last two equations of 6.8. It is easier to rename the last two equations of 6.8 (right side of the equations):

\[
k_4k_6 = \frac{\eta}{\beta - \delta} = A
\]  
\[
(k_4 + k_6) = \frac{\varepsilon - \gamma}{\beta - \delta} = B
\]  

(6.9)

The system in 6.9 admits two solutions, two couples of which are equal. Therefore, solutions for \(k_4\) and \(k_6\) are:

\[
(k_{4,1}, k_{6,1}) = \left( \frac{B + \sqrt{B^2 - 4A}}{2}, \frac{B - \sqrt{B^2 - 4A}}{2} \right)
\]
\[
(k_{4,2}, k_{6,2}) = \left( \frac{B - \sqrt{B^2 - 4A}}{2}, \frac{B + \sqrt{B^2 - 4A}}{2} \right)
\]

(6.10)

Now, if solution \((k_{4,1}, k_{6,1})\) is considered, the model parameters can be expresses as:

\[
K_1 = \alpha
\]
\[
k_2 = \left[ \delta - \frac{\beta}{\alpha} \right]
\]
\[
k_{3,1} = \frac{1}{\sqrt{B^2 - 4A}} \left[ -\left( \frac{\gamma}{\alpha} - A \right) + \frac{B + \sqrt{B^2 - 4A}}{2} \left( \frac{\beta}{\alpha} - B \right) \right]
\]
\[
k_{5,1} = \frac{1}{\sqrt{B^2 - 4A}} \left[ \left( \frac{\gamma}{\alpha} - A \right) - \frac{B - \sqrt{B^2 - 4A}}{2} \left( \frac{\beta}{\alpha} - B \right) \right]
\]
\[
k_{4,1} = \frac{B + \sqrt{B^2 - 4A}}{2}
\]
\[
k_{6,1} = \frac{B - \sqrt{B^2 - 4A}}{2}
\]

(6.11)

Otherwise, solution \((k_{4,2}, k_{6,2})\) gives the following set for model parameters:

\[
K_1 = \alpha
\]
\[
k_2 = \left[ \delta - \frac{\beta}{\alpha} \right]
\]
\[
k_{3,2} = \frac{1}{\sqrt{B^2 - 4A}} \left[ \left( \frac{\gamma}{\alpha} - A \right) - \frac{B - \sqrt{B^2 - 4A}}{2} \left( \frac{\beta}{\alpha} - B \right) \right]
\]
\[
k_{5,2} = \frac{1}{\sqrt{B^2 - 4A}} \left[ -\left( \frac{\gamma}{\alpha} - A \right) + \frac{B + \sqrt{B^2 - 4A}}{2} \left( \frac{\beta}{\alpha} - B \right) \right]
\]
\[
k_{4,2} = \frac{B - \sqrt{B^2 - 4A}}{2}
\]
\[
k_{6,2} = \frac{B + \sqrt{B^2 - 4A}}{2}
\]

(6.12)
APPENDIX B

Couples \((k_{3,i}, k_{4,i})\) and \((k_{5,i}, k_{6,i})\) have the physiological meaning of rate constants of \([\text{carbonyl} -^{11}\text{C}]\text{WAY-100635}\) flow from the Free compartment to the Specific and to the Non-Specific ones, respectively, and they have to be associated to kinetics of the tracer in each compartment that verify some trend criteria. In fact, we expect:

1. the free compartment kinetics, associated to parameters \(K_1\) and \(k_2\), to follow the trend of the plasmatic curve (e.g. a decreasing exponential or a sum of decreasing exponentials),

2. the non specific compartment, associated to parameters \(k_5\) and \(k_6\), that rapidly exchanges with the free pool, to have a fast rising and a fast decreasing trend, similar to the free kinetic.

3. the specific compartment, associated to parameters \(k_3\) and \(k_4\), to slowly rise until it reaches a plateau or it decreases. This pool kinetics is completely different from that of the other two compartments.

It is possible to notice that the two system solutions, 6.11 and 6.12, differ from one another for the fact that, while their values for \(K_1\) and \(k_2\) are identical, values for \(k_4\) and \(k_6\) are exchanged. That is:

\[
\begin{align*}
k_{4,1} &= k_{6,2} \\
k_{6,1} &= k_{4,2}
\end{align*}
\]

and, as a consequence, the correspondent values for \(k_3\) and \(k_5\) are swapped too.
6. Conclusions

Now, once the model is numerically identified, the compartment kinetics $C_f(t)$, $C_s(t)$ and $C_{ns}(t)$ (functions of estimated parameters) on a cartesian graph results in two possible set of kinetics, as in the example that follows:

The analyst has to choose which one of the two solutions of the system is associated to the correct physiological interpretation of the 3-TM model, that is, which one of the two sets of parameters $(K_1, k_2, k_{3,1}, k_{4,1}, k_{5,1}, k_{6,1})$ or $(K_1, k_2, k_{3,2}, k_{4,2}, k_{5,2}, k_{6,2})$ is able to assign the model the exact physiological meaning.

The correct procedure consist in choosing as $(k_3, k_4)$ the $(k_{3,i}, k_{4,i})$ values that originate a slowly rising and then a slowly decreasing curve and, as a consequence, in choosing as $(k_5, k_6)$ values that generate a curve much similar to the free kinetic.

In the example shown above, the correct solution for the system is the one represented in the left graph.

Figure 6.2: Compartment kinetics for the Three-tissue model.
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