Positron Emission Tomography Region of Interest and Parametric Image Analysis Methods for Severely-Lesioned Small Animal Disease Models

by

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Abstract

Small animal positron emission tomography (PET) image analysis can be particularly challenging with heavily-lesioned animal disease models with limited tracer uptake such as the 6-hydroxydopamine (OHDA) lesioned rat model of Parkinson's disease. Methodology-related variations in measured values of 10% or 15% can obscure meaningful biological differences, so accurate analysis methods are essential. However, placing regions of interest (ROIs) on these images without additional guidance is unreliable, and can lead to significant errors in results. To address this problem, this work develops a partly atlas-guided method place ROIs on structures that lack specific binding with presynaptic dopaminergic tracers. The method is tested by correlation of PET binding potential (BP) with autoradiographic binding measurements, and with repeated PET scans of the same subjects, both with the presynaptic tracer $^{11}$C-

When directly comparing PET images of the same subject to detect changes, it is essential to minimize variations due to analysis method. To this end, a masking method for automated image registration (AIR) of PET images with dopaminergic tracer rat images is developed. Coregistration with AIR and separate ROI placement are compared and tested with repeated scans of the same rat with DTBZ, and are found to be equivalent.

Kinetic modelling algorithms may also introduce bias or scatter to binding potentials (BP) calculated from TACs or in parametric images. To determine the optimal method for this step, algorithms for dopaminergic tracers are compared for small animal DTBZ, $^{11}$C-

Among the tested methods is a new variant of the Logan graphical kinetic modelling method, developed in this work, that is significantly less biased by target tissue TAC noise than the standard Logan approach. The modified graphical method is further compared with the Logan graphical algorithms with added-noise simulations. The simplified reference tissue model (SRTM) is found to have the best method for ROI TAC data, while the modified graphical algorithm may be preferred when generating parametric images.
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Chapter 1: Background

1.1 – Introduction

1.1.1 – PET Overview

Positron emission tomography (PET) is an imaging modality capable of detecting very small concentrations of radioactively-labelled chemicals in objects. This capability is particularly useful when used to measure concentration of biologically interesting radiochemicals in living organisms. Measuring the concentration of such radiochemicals allows the investigation of biochemical processes or physiological function of tissue in its natural microenvironment in vivo, rather than requiring excised samples or subject sacrifice when in vitro measurements are done. In vivo measurements are essential or very beneficial for medical imaging applications such as investigation of neurological disorders such as Parkinson’s disease and tumour detection and characterization in cancer patients. As well, investigation of neurotransmitter signalling and binding in vivo depends on the ability to measure concentration in living subjects.

PET measures appropriately-radiolabelled molecules: those that contain isotopes that decay by positron emission. Radioactive isotopes are atoms with numbers of nucleons that cause them to spontaneously decay into more-stable atoms. Some isotopes decay by emission of a positron, or beta-plus particle, and some of these can be used in PET imaging. Positron-emitting isotopes such as $^{11}$C, $^{18}$F, or $^{15}$O can be easily incorporated into biologically interesting molecules because their stable counterparts naturally occur in many such molecules. Consequently, these positron-emitting isotopes can be substituted for stable isotopes without significantly changing the altered chemical’s biological properties. As well, many positron-emitting isotopes, including these, have half lives measured in minutes. This is useful for PET because short decay times allows a relatively large level of radioactivity to be injected at the start of a scan, which will almost completely decay in a relatively short period of time. Sufficient radioactivity may be injected for scanning purposes without giving the patient or subject a large total radiation dose. As well, the radiological decay of these isotopes is of the same order of magnitude as the
biological decay rate – the rate at which the tracers interact in tissue, until a final stable condition is reached – making much longer scanning unnecessary.

For PET imaging to be useful, however, accurate image analysis methods are required, and improvements to analysis methods is the focus of this work. In particular, region of interest (ROI) placement on images, used to average image values within anatomical structures must be done carefully to produce reliable results that reflect actual biological changes, and not merely effects of inconsistent analysis. After placing ROIs, the averaged image data must be further analyzed to extract biologically relevant parameters such as the binding potential (BP) from images.

Various approaches exist to determine biological parameters from multi-frame "dynamic" PET images, and in this work several of these are evaluated. The commonly-used reversible graphical Logan method (Logan et al., 1996) is compared with the Gunn solution (Gunn et al., 1997) to the simplified reference tissue model (Lammertsma and Hume, 1996), the Ichise linearized full reference tissue model (Ichise et al., 2003), Holden’s expectation maximization impulse response method (Holden et al., 2007). Additionally, a modification of the Logan method is proposed and evaluated in comparison with the standard Logan method. Most comparisons in this work are done using rat PET images, however the modified graphical method comparisons also use human data. Comparisons are done with region of interest (ROI) time activity curve (TAC) data for all algorithms, and with parametric imaging in cases where doing so is feasible.

1.1.2 - PET Imaging

The PET imaging process begins with radioisotope generation. PET requires positron emitting isotopes, which have more protons (or fewer neutrons) than more-stable nuclei that they decay into; positrons are positively charged, and their emission converts a proton into a neutron. Proton-rich isotopes are generated with particle accelerators. The motion of charged particles and ions is affected by electric and magnetic fields, which are used to contain and accelerate them. When sufficient energy is obtained, they can overcome coulomb repulsion of
atomic nuclei, enter the nucleus and react with it. This produces new isotopes and elements from the target material.

Once generated, positron emitting isotopes must be bound to a suitable biologically interesting molecule before they may be used. Molecules with an attached radioisotope are referred to as radioactively labelled, or radiolabelled, and their presence may be detected in a PET scanner when the radioisotopes decay. Labelling involves chemically binding the radioisotope, which behaves chemically nearly identically to other more-stable isotopes of the same element, to the appropriate chemicals.

Because positron emitting isotopes are generated by adding protons to another element, nearly all atoms of the element being generated are radioisotopes. Other isotopes, which do not decay in the manner desired, are known as carrier. An amount of an element without any undesired isotopes is referred to as carrier-free. Having nearly carrier-free samples of the radioisotope being manufactured is very useful, as it allows the final product labelled molecule to have a very high specific activity, or activity per mass of sample. If a significant fraction of the produced element was the wrong isotope, it would be very difficult to separate out the desired isotope, and the final product would have a low specific activity, as both radioisotope and undesired isotopes will bond equally when tracer is being manufactured.

Once a suitable sample of radiolabelled chemical has been manufactured, it must be introduced into the system or organism being studied. Most such chemicals are produced as a solution in water, which may be diluted to the desired total volume, and then injected into the subject. Injections are frequently intravenous, though a muscular or subcutaneous injection may also be used. Other chemicals, such as oxygen-15, are gaseous and are delivered by inhalation. The following discussion will focus on injected tracers, but similar concepts apply for inhaled.

The total radioactivity of radiolabelled chemicals used in PET scans is typically quite small. The activity concentration in the body of an animal being scanned may be pico- or nanomolar. At concentrations this low, the total amount of chemical is small enough that the system being studied is not significantly affected by its presence. Chemicals at these concentrations are referred to as tracers. Because of the high sensitivity of PET scanning, it is
possible to use these extremely low concentrations and low total radioactivities and still generate useful images.

There are a variety of injection timecourses that may be used when doing PET scans. Common methods include continuous infusion, in which a constant flow of tracer is passed into the bloodstream throughout the scan, and the bolus injection, in which a single injection is given near the start of the scan. The specific timecourse of tracer concentration in the blood is important when analyzing the image data, and its measurement will be discussed later.

After injection, tracer chemicals circulate through the body of the subject. It is assumed that the blood mixes well and that the concentration in blood in different parts of the body becomes equal fairly quickly, and that there are not significant oscillations in concentration as different volumes of blood (such as the volume immediately before or after the volume passing through the point and time of injection) pass by sites of interest in the body.

As tracer in blood passes by such sites, its biologically-interesting characteristics become relevant. Tracers are taken up by binding sites or cellular transport mechanisms, and begin to localize in tissues at concentrations larger than that due to the blood. The way the concentration in tissue varies or accumulates with time is characteristic of that tissue and its interaction with the tracer. From the time-varying concentration profile, parameters that characterize the tissue may be calculated.

PET isotopes decay into more-stable isotopes by emitting a positron. Such a positron will travel a short distance, on the order of millimetres to centimetres, losing its kinetic energy in a series of small collisions and other interactions with the electrons and atomic nuclei. After losing almost all of its kinetic energy, the positron will annihilate with an electron in the material, producing two gamma rays with energy of approximately 511 keV. The PET scanner is designed to detect these gamma rays. A short (on the order of 4 to 6 ns) coincidence timing window is used by the scanner electronics to determine if two detected photons occurred simultaneously, and if so, the line between the two interactions is recorded has having one detected event.
Recording data with an object in a PET scanner that has radionuclides present in it is referred to as an emission scan. After an emission scan of sufficient time – typically minutes to hours – a large number of coincident events and their lines of response have been recorded. These data may then be reconstructed into 3D or 4D (3D space + time) images. With appropriate correction factors applied, this gives a 3D, possibly time-varying, measurement of the concentration of labelled tracer in the imaged object.

1.1.3 - Instrumentation

A standard PET scanner consists of multiple detector elements that are capable of detecting and characterizing positron decay gamma rays. The elements are spatially distributed to cover as large a solid angle as other design considerations allow, in order to maximize the number of lines of response between them, and to optimise the spacing of these lines. A conceptually simple PET system design has multiple stacked detector rings, or polygons, oriented perpendicular to and centred on a common axis, forming a (nearly) cylindrical shell. The cylinder axis is typically oriented horizontally, allowing patients or objects to be placed on a table or similar support structure to hold them in place in the scanner’s field of view after they are slid in.

Figure 1.1 has been removed due to copyright restrictions. The information removed is a photograph of a PET scanner from Vesna Sossi.
1.1.4 - Detectors

PET detectors must provide a variety of information about detected photons to generate useful images. In addition to detecting gamma rays, the system must provide adequate timing resolution to distinguish coincident gamma detections from unrelated detections that occur near each other in time, but not simultaneously. As well, good energy discrimination is required to reject as many scattered photons as possible, and spatial resolution must be sufficient to distinguish interactions in separate but tightly spaced lines of response.

The most commonly used detection apparatus in PET scanners is the combination of scintillating crystals and photomultiplier tubes (PMT). The crystals absorbs the annihilation photon and emit visible light, which interacts with a photocathode to emit electrons, which are amplified by the PMT and recorded as an electrical signal of magnitude proportional to the energy of the original photon. Less commonly used systems employ avalanche photodiodes in place of PMTs, or replace the entire system with a semiconductor detector (Pichler et al., 2006).

1.2 - Image Quality

1.2.1 – Quality Overview

To be useful, PET images must be sufficiently high-quality representations of objects that are imaged. Important measures of image quality are the spatial resolution and quantitation.

Spatial resolution is a measure of how well an image localizes features of an object. In particular, the point-spread function of an imaging system describes how a physical small isolated point-source-like object will appear in images. If spatial resolution is poor, a small point-like object will appear in images as a spread-out distribution of non-zero voxel values, and multiple adjacent small objects may be indistinguishable from a larger single object. Factors that affect spatial resolution include non-collinearity of annihilation photons, range of positrons between emission from a decaying radionuclide and annihilation, as well as the finite size, number and spacing of detector elements in the scanner.
Quantitation is how well the values in an image, in counts per voxel, match the radiotracer concentration, in Bq/ml, in the object being imaged. For PET imaging, the counts per voxel should match the tracer concentration in the corresponding volume of the object. Although there are some medical uses for non-quantitative imaging, many research applications of PET depend on accurate tracer concentration measurements. Various factors affect quantification, including attenuation of annihilation photons in the object, scattered photons, and the spatial resolution of the scanner. It is necessary to correct for these effects to achieve good quantitation.

### 1.2.2 - Attenuation

Photons may be Compton scattered when passing through an object. Because PET measures the concentration of a tracer by detecting photons produced by annihilations within an object, detected photons must have passed through some portion of the object to reach the scanner. Annihilation photons that are scattered in such a manner that they are not detected are considered to contribute to attenuation. Typically, this will occur when a photon is scattered into a path that takes it out of the scanner in a direction which does not pass through the detector ring. It is also theoretically possible for annihilation photons to interact by photoelectric absorption, which would also be considered an attenuated photon. In practice however, the cross section for photoelectric absorption of photons with energies near 511 keV is quite small, and almost all attenuation in PET is due to Compton scatter.

Photon attenuation can affect the final PET image in several ways. Most directly, annihilation photons produced during an emission scan – when the tracer concentration is being measured – may be attenuated. As all positron annihilation photons have nearly equal energy, 511 keV, the object’s material properties determine the degree of attenuation in different regions within the object. In objects with uniform activity distribution and attenuation, the uncorrected effects of attenuation cause the detected radioactivity in the centre of objects – where photons on lines of response must traverse the most material – to be lower than in areas nearer the edges of the objects. In more complicated objects, such as animals or humans, denser tissues such as bone can create shadows in some lines of response, reducing the apparent activity in structures near them.
1.2.3 - Attenuation Correction

PET scans record coincidence events, in which two photons are detected simultaneously, and these photons are emitted from the same point and travel in opposite directions. Whether or not each photon from a positron annihilation is attenuated is independent of the fate of the other photon, so the total probability of either or both photons being attenuated is the product of each being attenuated separately. Since the photons travel in opposite directions, their combined paths through the object trace a single straight line of response completely through it. The total probability of attenuation along that line is an integral of the attenuation factors along it, and can be thought of as a property of that line of response for a given object.

Line of response attenuation factors can be measured with a PET system by performing a transmission scan, in which a point source is moved around the subject in the scanner, and the transmitted intensity for each line of response is measured. This intensity is compared with a blank scan, which is a transmission scan done without an object in the scanner. The ratios of transmission to blank intensity give the attenuation of the object for each line of response. Having the attenuation factors of each line allows the weighting of those lines in the final image to be adjusted to counteract the effects of attenuation on final image quantification.

1.2.4 - Emission Scatter and Random Coincidences

Compton scattered photons may be deflected to a path that passes through the detector elements, unlike attenuation-causing scatter that deflects photons out of the scanner. The line of response for a pair of detected photons where one of those photons has been scattered will not be representative of the positron annihilation position. Scatter where one of the two photons undergoes Compton scatter, but is still detected, is the most important component of scatter in most PET systems; the scatter fraction of detected events (scattered / (scattered + unscattered)) can be as high as 70% for human whole-body imaging, or 30% for small animals in dedicated small animal PET scanners, which can significantly degrade image quality.
Alternatively, more than one separate decay event can occur simultaneously, or close enough that the photons are detected within the timing window of the detector system. Coincidences from this process are referred to as randoms, or random coincidences. Like emission scatter, their lines of response are essentially unrelated to the object’s activity distribution.

1.2.5 - Transmission Scatter

Scatter also affects the accuracy of transmission scan attenuation corrections. Singles mode transmission scans, where single photon interactions are measured without requiring coincidences, are typically performed with a point or line source at the radial outer edge of the scanner. Multiple lines of response that intersect this point source, through a range of angles, are simultaneously measured, allowing relatively fast data collection.

A problem arises with singles mode geometry, however, because photons initially travelling along one line of response may be scattered into detectors that are meant to measure the attenuation along another line of response. This can cause lines of response through the centre of objects to have extra photons recorded which were scattered out of adjacent lines. Erroneously high numbers of photons are measured, which leads to erroneously low attenuation values for the lines of response which receive excess scattered photons. That can be avoided by using shielded sources which emit photons in only a small angle, in order to measure attenuation along only one line of response at any time, however this greatly increases the time required to perform transmission scans.

1.2.6 - Scatter Correction

The number of scattered photons recorded by a PET scanner may be reduced by using an energy window to restrict the energies of detected photons that are accepted. Compton scatter is inelastic; photons that are deflected lose energy, with more energy lost at larger scattering angles. If the detector system has adequate energy resolution, a tight energy window around the 511 keV annihilation peak for emission scans will isolate photons which have not been scattered. For transmission scans, a similar peak around the energy of the photons may be used.
In practice, detector system energy resolution is at least 20% of the detected photon energy, and detected photons may appear to have an energy within a distribution around their true energies. If an extremely tight energy window is used, many non-scattered photons may be discarded if their estimated energy was outside the window. If too many detected events are discarded, the sensitivity of the PET scanner will be significantly reduced, producing poorer quality statistics or requiring longer imaging times. Instead, a somewhat wider energy window than might otherwise be ideal is used, in order to collect enough counts to produce adequate imaging statistics. This will inevitably cause some scattered photons to be included in the recorded events.

For transmission scans, a collimated source may be used to emit photons confined to a tight beam. Only photons detected in a single detector or small region of detectors are then counted. Any scattered photons deflected out of the beam and away from the active detector will not counted, and no photons exist to be scattered into the active detector from other original paths. This method greatly increases the duration of transmission scans, however.

Transmission scatter may also be modelled physically to estimate the contribution of scatter to the measured transmission values. With an iterative reconstruction algorithm, this allows singles mode transmission data to be significantly improved (Vandervoort and Sossi, 2007).

Emission scatter may be similarly modelled. Using the attenuation map generated from the transmission scan to estimate the scatter contribution to the measured emission data, emission data can be corrected for scatter, either with a direct subtraction method, or by including a scatter estimate in an iterative reconstruction method. (Watson et al., 2004)

1.2.7 - Non-Colinearity

Positrons propagating through a material may lose their kinetic energy and form an exotic atom of positronium with an electron from the material, and then annihilate with relatively little excess energy. Alternatively, the positron may annihilate with an electron without forming
positronium, while still retaining some kinetic energy. A positron with kinetic energy also has a nonzero momentum, and after annihilation, the emitted gamma rays will be non-collinear (typical angles of half of one degree) due to momentum conservation. In this case, after propagating to the PET detector ring, the line of response between the two recorded events will not intersect the point where the annihilation occurred. This will displace the line of response from the true annihilation position, causing blur in a PET image.

For a given PET scanner, improving or eliminating non-colinearity of annihilation photons is impossible. Changing the scanner geometry, by using a smaller-bore PET scanner limits the distance that photons travel before detection, can reduce the distance between the detected line of response and the true annihilation location, however. Alternatively, non-colinearity may be modelled and corrected with an iterative image reconstruction algorithm.

1.2.8 - Positron Range

When a positron is ejected from a decaying radionuclide, it is given some kinetic energy. Before the positron can annihilate with an electron, it loses most of this kinetic energy in small collisions with electrons and nuclei in the material. While losing this energy, the positron travels some distance in the material away from the location where it was ejected. As a result, the point where the positron annihilates, and thus the point where the annihilation photons originate and the line of response into which a PET system will assign a count if the annihilation photons are detected are spatially separated from where the tracer molecule was actually located. This introduces a degree of blur to PET images, limiting their theoretical resolution.

Each PET isotope ejects positrons with a distribution of kinetic energies, with, for example, most-probable energies near 200 keV for $^{18}$F and 400 keV for $^{11}$C, and maximum energies 631 keV for $^{18}$F and 958 keV for $^{11}$C. Positrons are light charged particles, and propagate through solid material in a manner similar to electrons. Such particles undergo many small collisions with electrons in the material, transferring small amounts of energy to those electrons, and Bremsstrahlung interactions with atomic nuclei, which cause a distribution of photon energies to be emitted and also deflect the particle. The net result of the distribution of
initial energies and frequent deflections is that the final annihilation location of the positron can be described as a spatial distribution around the ejection location.

For most PET scanners, little can be done to reduce positron range for a given tracer. Specialized PET scanners that function in high magnetic fields are an exception, because the magnetic field causes charged particles' paths to curve in a plane perpendicular to the field, reducing its range. Combined PET-MRI machines are being investigated which, to enable MRI, have such magnetic fields in place. For most dedicated PET scanners, however, the only correction for this effect is modelling as part of an iterative reconstruction algorithm.

1.2.9 - Detector Elements

PET scanners have an inherent resolution limitation due to the finite size, distribution and number of their detector elements. Lines of response exist only between detector elements, so separating and distinguishing objects in the field of view that are of comparable size and separation as detector element spacing is difficult. The result of this limitation is blurring of small objects. Some reconstruction algorithms model this blurring, but it is impossible to fully correct for this blurring, as it is a fundamental limit in the measuring capability of the system; regardless of the reconstruction method use, some fine details of objects cannot be distinguished by the measurement system.

1.3 - Image Reconstruction

1.3.1 – Reconstruction Overview

A PET detector records counts on each line of response. In order to estimate the concentration of tracer in a volume within the object being imaged, the line of response data must be reconstructed into a 3D image, or 4D image if multiple time-frame images are separately reconstructed of the same object. Various algorithms exist to perform reconstruction, including filtered backprojection, and the various iterative reconstruction algorithms.
1.3.2 - Filtered Backprojection

Backprojection is an image reconstruction algorithm in which count totals associated with PET lines of response are treated as integrals, or sums, of the activity in the object along the line. Essentially, the activity along each line has been projected into a single summed value for that line. If lines of response are uniformly sampled from all angles around the object, there is sufficient data for an image to be accurately reconstructed by projecting the line of response sums into the image volume, in a process referred to as backprojection. In this procedure, the values of the summed projections are smeared along the line of response, adding to all image voxels through which the line passes. Each voxel will be intersected by many lines of response, and all will contribute to the total intensity of the voxel. If there is not uniform sampling of angles around the object, then this procedure cannot be used, as the missing data invalidates the mathematic basis for backprojection and produces large image artifacts.

Simple backprojection is insufficient however, as the process adds a blurring to the image which is mathematically described in position space as “one over r”. That is, the blur is similar to that caused by a kernel inversely proportional to radial distance convolved with the image. Correcting for this blur can be done by filtering the image in spatial frequency space with a “ramp filter”. The ramp filter reduces lower spatial frequency components of the image, with a linearly increasing value until a maximum cutoff frequency. The maximum cutoff is required because at higher spatial frequencies than the PET system can resolve, due to the limited sampling and associated Nyquist frequency, the only signal components are noise. Magnifying noise degrades image quality. As well, aliasing of high-frequency components of projections when backprojecting into an image will become much more apparent if those high-frequency components are magnified.

This algorithm has the useful property that the images it produces are a linear function of the measured data. That is, if two sets of measured data are summed before reconstruction, the resulting image would be equal to the sum of the separate data sets’ reconstructed images.

Overall, filtered back projection is fast and convenient, but can be severely limited. It is necessary that the measured data have uniform angular sampling, which is not possible with
some PET scanners. As well, the filtered backprojection algorithm has limited ability to add correction factors for scatter, positron range or annihilation photon noncollinearity, unlike many iterative algorithms. The PET system used in this work was the Siemens/Concorde Focus 120, which does provide uniform angular sampling, and filtered backprojection was used to reconstruct PET images.

1.3.3 - Iterative Reconstruction

When filtered backprojection cannot be used, iterative reconstruction algorithms may be used instead. Alternatively, iterative reconstruction may be used even when filtered backprojection is possible, because iterative methods may incorporate correction factors that backprojection cannot. The microPET system used in this work is capable of reconstruction by backprojection, however Siemens/Concorde also provides iterative reconstruction software with the scanner.

A commonly-used type of iterative reconstruction algorithm is expectation maximization. The maximum likelihood expectation maximization (MLEM) variation incorporates the statistical distribution of radioactive measurements – the Poisson distribution – to improve reconstruction results. Multiplicative correction factors are applied iteratively to improve the estimated image. The ordered subsets expectation maximization (OSEM) variation uses only a subset of the available projections on each iteration, and converges slightly faster than single-set MLEM. (Hudson and Larkin, 1994)

Unlike backprojection reconstruction, iterative techniques are not linear. In general, the reconstruction of the sum of two data sets will not equal the sum of those data sets’ separate reconstructions. Consequently, for a given acquired data set, the reconstructed image will depend on the specific parameters of the reconstruction algorithms, including number of subsets or the number of iterations.
Chapter 2: Parkinson's Disease Rat Model and PET Tracers

2.1 - Neurotransmitters

2.1.1 – Neurotransmitters Overview

Neurotransmitters are chemicals used in the body to convey information or signals between nerve cells, or neurons, and other cells (which may also be neurons). Neurotransmitters are used to convey signals across synapses, which are the gaps separating the neuron in which the signal originates and the cell to which it is conveyed. Presynaptic neurons synthesize or store neurotransmitters, and release them into the synapse when suitably stimulated. The postsynaptic cell membrane along the synapse has receptors which detect the neurotransmitter, and convey information about its presence into their cell.

2.1.2 - Synaptic Signalling

Neurotransmitters synthesized in presynaptic neurons and stored in synaptic vesicles in these neurons. When a signal of sufficient strength excites the presynaptic neuron, vesicles merge with the cell wall at the synapse, releasing the transmitter into the synaptic gap, or cleft. Receptors on the postsynaptic neuron's cell wall along the cleft detect the sudden spike in transmitter concentration when the receptors and transmitters chemically bind to each other. Signals propagated by synapses may be excitatory or inhibitory, with the former causing the postsynaptic cell to increase its activity, and the latter causing the postsynaptic cell to be less active.

After a signal causes release of neurotransmitter into the synaptic cleft, the transmitter that was in the presynaptic vesicles must be replaced for the presynaptic cell to be able to convey another signal. The presynaptic neuron has neurotransmitter-transporters on its cell membrane at and near the synapse. These transporters take up the transmitter from the synaptic gap and the surrounding extracellular fluid – to which transmitter may have diffused out of the synaptic cleft – and store the tracer back in vesicles for future release.
2.1.3 – Striatal Dopaminergic System

A group of neurons originate in the substantia nigra, and extend axons into, or innervate, the striatum. These presynaptic neurons form connections to postsynaptic neurons within the striatum. The neurotransmitter dopamine (DA) is synthesized in these presynaptic neurons, and stored at high concentration in specialized vesicles near the synapse. To propagate signals, the presynaptic neurons generate large spikes in neurotransmitter concentration in the synaptic gap by merging vesicles with the cell membrane at the synapse, expelling their contents of highly concentrated neurotransmitter.

Synthesis of neurotransmitter does not occur in vesicles, and reuptake of transmitter after a vesicle's contents are released in the synapse moves the transmitter into the cytoplasm, not directly into the vesicles. In order to move transmitter molecules into the storage vesicles, the vesicle membranes have proteins referred to as vesicular monoamine transporters (VMAT), which move dopamine from the cytoplasm into the vesicle. There are multiple types of VMAT, including the VMAT-2.

After release into the synapse, spikes in dopamine concentration are detected by receptors on the membrane of the postsynaptic neuron. One such receptor is the dopamine D2 receptor, which responds to a spike in dopamine concentration by propagating an inhibitory signal.

After release and detection, high concentrations of dopamine in the synapse begins to diffuse out of the synaptic gap. To recover some of dopamine, and to reduce the concentration in the synapse before future signals can be propagated by additional concentration spikes, the dopamine is transported back into the presynaptic neuron. Proteins that move dopamine from the synapse into the presynaptic neuron are the membrane dopamine transporters (DAT).
Figure 2.1 has been removed due to copyright restrictions. The information removed is a labelled diagram of the human brain from David Holland.

Figure 2.1 - Human brain section with labelled substantia nigra and striatum. Red arrows indicate path of nerves projecting from substantia nigra into striatum which propagate signals using dopamine. (David Holland, www.swmed.edu)

Figure 2.2 - Pre- and post-synaptic neurons with locations of VMAT-2, DAT and D2 receptors.

Figure 2.2 - Pre- and post-synaptic neurons with locations of VMAT-2, DAT and D2 receptors.

2.2 – Parkinson's Disease Rat Model

2.2.1 – Parkinson's Overview

In Parkinson’s disease, accepted theory indicates that the dopaminergic presynaptic neurons discussed above begin to die. There are fewer neurons able to propagate individual signals from the substantia nigra into the striatum, and there is a generally reduced amount of
dopamine in the striatum. Reduced dopamine is believed to be related to the Parkinsonian motor and, in some cases, cognitive symptoms.

2.2.2 – Rat Model

The origin or cause of Parkinson's disease and its progression are presently not fully explained. Animal models, including rats, are used to investigate the mechanisms of the disease.

This work primarily focuses on analysis of PET images of rat models of Parkinson's disease (PD). The currently most commonly used model of PD is lesioning with 6-hydroxydopamine (OHDA). Sprague-Dawley rats were unilaterally lesioned in medial forebrain bundle with 6-hydroxydopamine. This neurotoxin causes a severe but selective loss of dopamine-releasing nerve terminals in the striatum (Debeir, 2005). OHDA lesioning is not progressive; rats' symptoms and measurable lesion severity do not significantly and consistently change with time after the initial response to the procedure. The induced lesioning is nevertheless used to mimic the progressive loss of these neurons in human Parkinson's patients. Unilaterally lesioned rats exhibit unilateral Parkinsonian symptoms, and are considered to be a useful tool to study the condition.

2.2.3 - Tracers Used

In this work, three neurotracers are used. $^{11}$C-dihydrotetrabenazine (DTBZ) binds to the monoamine vesicular transporter type 2 (VMAT-2), which are proteins responsible for transport of dopamine into synaptic vesicles. In this work, $[^{11}\text{C}](+)-\alpha$-dihydrotetrabenazine was used. Pharmacologically, tetrabenazine-like molecules bind to VMAT and inhibit transport of DA from cytoplasm into storage vesicles, allowing it to break down in cytoplasm. At tracer concentrations, such as those used in PET imaging, there is too little DTBZ present to significantly affect DA transport, however.
Figure 2.3 - DTBZ PET image of unilaterally lesioned rat model of Parkinson's disease in transaxial (left), coronal (centre) and sagittal (right) plane orientations. In the transaxial and caudal planes, high concentration of DTBZ can be seen in the unlesioned left striatum, whereas very little uptake is seen in the heavily-lesioned right striatum.

\[ ^{11} \text{C}-\text{methylphenidate (MP)} \] binds to membrane dopamine transporter (DAT), which are proteins responsible for transport of dopamine from a synapse back into the cytoplasm of the presynaptic neuron. Pharmacologically, MP acts as a dopamine reuptake inhibitor, by blocking DAT sites from binding to DA in the synapse. Again, at tracer concentrations, this effect is negligible.

Figure 2.4 - MP PET image of unilaterally lesioned rat model of Parkinson's disease in transaxial (left), coronal (centre) and sagittal (right) plane orientations. In the transaxial and caudal planes, slightly higher concentration of MP can be seen in the unlesioned left striatum, whereas less is seen in the heavily-lesioned right striatum.

\[ ^{11} \text{C}-\text{raclopride (Rac)} \] binds to postsynaptic D2 receptors, which are responsible for reacting to the presence of dopamine in the synapse and propagating signals to the postsynaptic neuron. Pharmacologically, it is a competitive antagonist for D2 receptors, blocking DA binding (Debeir et al., 2005), but at tracer concentrations, there is too little Rac present to significantly affect normal brain chemistry and signalling.
These three tracers – DTBZ, MP and Rac – are used to investigate mechanisms involved in Parkinson's disease; the combination of tracers allows theories of disease mechanisms and compensation to be investigated in ways that a single tracer could not. For example, Rac scans can be performed before and after administration of levodopa (L-DOPA), a synthesis precursor to dopamine. A large loss of Rac binding to D2 receptors after L-DOPA is given indicates more DA (produced from the L-DOPA) in the synapse competing for the receptor sites, due to lack of DAT proteins to take DA out of the synapse. Similarly, both DAT and VMAT-2 receptors are presynaptic, yet differences in their tracer binding after lesioning, compared to unlesioned striata, and in Parkinsonian human patients may be observed. It is theorized that DAT are downregulated in surviving presynaptic dopaminergic neurons after some neurons are lost, as a compensatory mechanism to increase the amount of DA in synapses. (Lee at al., 2000)
Chapter 3: ROI Placement

3.1 – Analysis Introduction

3.1.1 - Analysis Overview

There are two main methods to analyze 4D (3D volume + 1D time) rat brain PET data of tracers such as DTBZ, MP or Rac. The time activity curve (TAC) of each voxel can be analyzed separately, producing an estimate of the biological parameter of interest for each. Parameter estimates for each voxel can be displayed as a parametric image, with the parameter estimates in place of measured voxel values. Alternatively, a volume of pixels’ TACs may be averaged into a single TAC that is taken to be representative of the entire volume. Averaging is done by drawing or placing volumes on the PET data in which the individual voxel TACs are to be combined. If a single volume is placed across multiple image planes, the volume is referred to as a volume of interest (VOI). If separate areas are placed on each image slice, and then combined to form the final volume, the individual areas are referred to as regions of interest (ROI). The following will focus on ROI placement, as this was the method used in this work.

When available, anatomical information from magnetic resonance image or x-ray computed tomographic images can be used to guide ROI placement. Acquiring these data may not be possible or practical in all cases, however, due to cost, time limitations and in the case of x-rays, unjustifiable additional radiation exposure. As well, there are presently few or no scanners capable of simultaneously acquiring PET and CT or MRI images. Instead, separate scans are taken of the same object, which requires the PET and anatomical information to be coregistered before the latter can be used to guide ROI placement on the former. Unfortunately, multimodality coregistration can be imprecise and difficult.

In many cases, including placing ROIs on rat brain PET images examined in this work, it is necessary to use only the values of the PET image voxels to guide ROI placement. Depending on the particular tracer used, image quality, and biological state of the object that was imaged, this may be sufficient, or it may be nearly impossible to do reliably. Difficulties arise when the structure(s) of interest in an investigation do not appear prominently in the PET images being
analyzed. In this case, the unaided placement may be little better than guesswork, perhaps
guided by experience with similar images or other landmarks within the image.

3.1.2 - DTBZ PET Rat Brain ROIs

In this work, methods were developed to improve the ROI placement on PET images of
the tracer DTBZ in unilaterally lesioned rat brains. This method is also applicable to other
similar tracers. The structures within the brain on which ROIs were placed were the lesioned
and unlesioned striatum, and the cerebellum. DTBZ is strongly taken up by unlesioned striata,
and appear prominently in PET images, where the surrounding brain takes up very little.
Lesioned striata take up less DTBZ than unlesioned, depending on the severity of the lesion, and
very heavily lesioned striata can be indistinguishable from the background brain regions. The
cerebellum cannot be seen in DTBZ PET images, as it appears similar to heavily lesioned
striatum. It is necessary, for the cerebellum and lesioned striata, to have additional guidance to
place ROIs reliably.

![Figure 3.1 - DTBZ PET image of unilaterally lesioned rat brain. Transaxial slices through the striatum (left) and
cerebellum (right) show ROIs placed over these structures, with lack of structure in images to indicate their
locations.](image)

3.1.3 - Previous Methods

Despite this necessity, the methods used for rat brain analysis prior to this work were
inadequate. Analysis was done with the ASIPro image manipulation program that is provided
with the Siemens / Concorde Focus 120 microPET system. Hour-long PET scans were
reconstructed into 128x128x95 voxel volumes, with 17 volumes, or time-frames, per dynamic
4D PET image. ASIPro allows one to view PET images taken with the microPET system, draw,
edit, save and reload ROIs, extract time activity curves (TACs), and perform some image manipulation such as summing multiple frames' volumes.

Circular ROIs, approximately 8 pixels or 6 mm\(^2\) in size, were placed on five PET planes of the unlesioned striatum. Based on experience of the investigator with the approximate relative locations of the two striata, another set of same-sized circular ROIs was placed over the best-estimate location for the lesioned striatum. The lesioned striatum itself was not visible, as the lesioning causes the tissue to not take up DTBZ.

Cerebellar ROI placement was more difficult than striatal ROIs. There is no visible structure in a DTBZ PET image near the cerebellum, and the cerebellum itself does not appear in the image as a distinct feature. To determine the approximate location of the cerebellum relative to the easily-identified unlesioned striatum, a rat brain atlas was manually registered to the PET image. The atlas used was created by Toga et al. (Toga et al., 1995) by freezing and sectioning a rat brain. The sections were photographed, and the resulting images were reassembled into a 3D volume. The volume was then segmented into categories for various brain structures such as the striatum, cerebellum, hippocampus, and cerebral cortex. This atlas was generated from one Sprague-Dawley rat brain, but it has been shown that rat brains are anatomically very similar over a range of rat body weights, so this atlas should be adequate for analysis of other rats' brain images.

Figure 3.2 - Three-dimensional rat brain atlas by Toga et al., shown in sagittal (left), transaxial (centre) and coronal (dorsal-ventral, right) plane sections. Structures seen with different colours have been segmented and labelled with distinct voxel values.

Atlas registration to PET was done by entering translation distances, using the RView program (http://rview.colin-studholme.net/). RView is able to draw an isovalue contour of an
image and superimpose this over another image. In this case, the contour of the atlas was drawn over the PET data. After the two were registered, the distance between the cerebellum in the atlas and the striata in the PET image was measured, and this distance was converted into a number of PET image planes separating the two structures.

The appropriate number of planes was counted in ASIPro from the striatal ROIs, to determine the image planes where cerebellum ROIs should be placed. Three elliptical ROIs, approximately 22 pixels or 16 mm$^2$ were placed on the cerebellum. The location of these ROIs within the predetermined planes was determined by best guessing an appropriate location, based on any discernable structure to the image, and distance from the visible body surface.

This method of ROI placement was not reliable. Particularly for lesioned-side striatal values, but also for unlesioned values, final results of analysis seemed to suggest that heavily-lesioned rats could not be analyzed. After observing this procedure, it was concluded that an improved method was necessary.

3.2 - Image Coregistration

3.2.1 – Coregistration Overview

The first method used to improve DTBZ PET rat brain analysis is image coregistration. Coregistration involves reslicing one image so that its voxel values are resampled in such a way that the contents are spatially matched, in the image, with the contents of the target image. This method can be used when directly comparing multiple images, ideally of the same animal. This situation arises when comparing results of scans taken before or after a treatment or intervention is performed on an animal.

If images can be accurately coregistered, then it should be possible to use the same set of ROIs on both the resliced and target images. There may still be inaccuracies in the absolute values of the results, as the ROIs will not be in the correct locations on either image if they are placed incorrectly on the registration target and then copied to the resliced image. However, in this case, any variation in the calculated results between the two images will be caused only be
the variations in the images themselves. This should allow the two images to be compared, without ROI placement inconsistency affecting the results.

There are numerous algorithms used for medical image coregistration. Intramodality registration is used when the images being coregistered are acquired with the same imaging modality. Modality in this case refers to the combination of equipment used, such as a PET or MRI scanner, and the specific details of the scan, such as the tracer or condition of the subject or object characteristic being imaged. Intermodality registration is also performed, where the images being registered are taken with different modalities, such as MRI to PET or multiple PET scans taken with different tracers.

3.2.2 — Registration Algorithms Overview

Numerous algorithms have been developed for image registration. Most methods are a variation on the theme of optimizing a cost function that estimates how well images are registered. Each method has a different cost function, based on different assumptions or theoretical basis. Optimization finds the translation, rotation, skews, scale factors or more complicated spatial transformations that map one image’s coordinates onto another image’s coordinates. For PET to PET coregistration done in this work, six-parameter rigid transformations were used, in which only three-axis translations and three-axis rotations were used. This limitation is suitable for the PET-PET coregistration because the images being coregistered are generally of the same object, or very similar objects, and no more-complicated types of transformations would be expected to apply. Registration algorithms investigated in this work include the mutual information maximization method, automated image registration (AIR), and various options available in the minitracc program provided by the Montreal Neurological Institute’s MINC software system.

3.2.3 — Mutual Information

Mutual information (Wells et al., 1996) is a registration algorithm used primarily for intermodality registration, though it can also be applied to intramodality PET to PET registration. Mutual information attempts to maximize the degree to which one image’s voxel
values can predict the other image’s voxel values. Consequently, the mutual information between two images is maximized when there are many valued voxels with similar values in one image which overlay many voxels with (potentially different) similar-valued voxels in the other image.

The values of individual voxels in an image can be viewed as random samples from a probability distribution that characterizes that image. Mutual information can be thought of in terms of the joint probability distribution of two images’ voxel values. In this view, the joint probability distribution is a two-dimensional surface with axes of the two images' voxel values and "height" at any given point proportional to the number of overlaid pairs of voxels with that point's pair of values.

For example, if image A has many voxels with value 5 that overlay voxels in image B with value 10, there will be peak in the images' voxel value joint probability distribution near the point \((A = 5, B = 10)\). If voxel values in both images are spread over a wide range, so that not all of image A's voxels have values near 5, and not all of image B's voxels have values near 5, a peak near the point \((A = 5, B = 10)\) provides mutual information between the images. This can be seen by noting that any given voxel in image B with value 10 is likely to overlay an voxel in image A with value 5, and that the converse is also true. If, however, all of image B's voxels were near value 10, then knowing the value of a voxel in image B provides no information about image A's overlaid voxel values, and there is little mutual information.

Mutual information is useful for intermodality registration because it can detect associations between groups of voxel values in one image with groups of different voxel values in another image. In the case of registration of MRI and PET images, a particular tissue might appear brightly in a PET image, and be dark in an MRI, and another tissue might appear at moderate brightness in both. For PET to PET registration, two images of the same subject, tracer and scanning conditions will be expected to have similar voxel values in voxels within the same anatomical structure. In either case, a mutual information cost function will be optimized when the two images are registered, because many voxels with corresponding values in both images will be overlaid, and few voxels with the same values will be overlaid on non-corresponding values in the other image. The results of MRI-PET and PET-PET may differ,
however, because the intermodality joint probability distribution will be expected to have discrete peaks, whereas the intramodality distribution may have a continuous ridge where adjacent values in one image tend to overlay adjacent values in the other image. It may be the case that mutual information is non-optimal for PET to PET registration, as it does not make and use the assumption that voxel values in well-registered images are linearly correlated, and only assumes that they have the described discrete peaks in their joint probability distribution.

The implementation of mutual information in the RView program was tested. This algorithm was unstable, and prone to crashing when run. Further, when it ran to completion, the results were unsatisfactory, with gross misregistration errors commonly observed, for both PET to PET and brain atlas to PET purposes.

The MINC software package also has an implementation of mutual information as one of its cost functions in its minctracc program. This functionality could not be used, however, as minctracc was also unstable and prone to errors when using non-trivial registration functionality.

3.2.4 – Automated Image Registration

The most successful algorithm tested in this work was automated image registration (AIR), created by Woods et al. (Woods et al, 1992). The standard intramodality version of AIR attempts to minimize the variance of the ratio of overlaid voxel values in the images being coregistered. In cases where the images being registered are of the same modality, it is generally reasonable to expect that scans will have a proportional relationship in voxel values across all tissue types and anatomical structures in the object. When images are well-registered, there should thus be little non-statistical variance in the ratio of overlaid voxel values.

The implementation of AIR provided by Woods was tested, and found to produce good results, if properly used. As discussed below, the assumption that images of the same modality will have a single voxel value ratio when properly registered depends on there being a single correct registration transformation for the whole image volume. For images of soft tissues which may move relative to each other between images, a single transformation may not suffice. Instead, it may be necessary to restrict the registration calculation to consider only a subset of the
image volumes, in which a single transformation is adequate. Alternatively, a higher-order registration algorithm could be used, which allows deformation of the spatial relationships between anatomical structures in an image such as a rat head and upper torso, however this method was not investigated in this work.

The MINC software package program minctracc also provides a variance of voxel value ratio cost function, however the same issue as discussed for mutual information prevented its practical use.

3.2.5 — Masking with AIR

While AIR was found to be the most useful and accurate registration algorithm of those tested, it is not trivial to use successfully for DTBZ PET rat brain images. It is assumed when using the AIR algorithm that a constant linear relationship will relate voxel values when the images are well-registered because the two images are taken of the same object in similar conditions. When PET scanning animals, rats in particular, this assumption is not true for the entire volume of images. Rather, there are numerous soft tissues in rats which are able to move relative to each other between PET scans. It was frequently observed that the structures in the necks of rats, which strongly take up DTBZ and thus appear prominently in PET images, were moving from scan to scan. As well, the mouth of the rats could be open or closed during scans, and the bulk of the rat’s body could be differently positioned. Using the AIR algorithm on the entire image volumes generally did not produce good registration results.

Figure 3.3 - DTBZ PET rat head images showing significant movement of tissues relative to brain between scans.

When analyzing rat brains by coregistration and shared ROIs, it is only important that the brains be properly registered within the volume of the brain. If there are soft tissue movements
between the scans relative to the brain in each, it is expected that there will be significant
differences in the appearances of the images in these regions, even when the brains themselves
are well-registered. This distinction is mirrored in the motivation for brain masking when doing
registrations. Since the only regions of the PET images that are expected and desired to be
registered are the brains and any tissues that are rigidly fixed relative to the brain, any other
regions that are expected to move relative to the brain should be excluded from the AIR ratio
variance calculation.

The AIR algorithm (and various other registration algorithms) include the ability to use a
mask image to limit the voxel of the resliced and target images which are considered in the
alignment calculation. Mask images typically have nonzero voxel values where the resliced and
target images are to be included in the alignment calculations, and zero values where the resliced
and target images are to be ignored. Using an appropriate mask image allows the desired regions
— those rigidly fixed to the brain — to be included in AIR alignment calculations, which omitting
undesired regions, significantly improving registration results.

When using masks to do AIR registration, it was also found to be helpful to use
appropriate thresholding of image voxel values. Thresholding acts similar to masks, in that it
excludes some voxels from the registration calculation. Rather than including or excluding
based on another image, however, thresholding includes or excludes voxels based on their own
values. In general, thresholding in this context is used to remove lower-valued voxels, such as
those of the bulk a rat’s body in a PET DTBZ scan, while including the higher activity regions
that are of interest. When using masking, appropriate thresholding may also help eliminate
sensitivity of the final registration result to the specific position of the mask. This is presumed to
occur, though is difficult to verify, because appropriate masking is able to remove all voxels of
low value that are located near the edges of the mask-included area. If no voxels are included
near the edges of the mask regardless of the mask’s position, then small changes in mask
position cannot affect the final registration results. It is helpful to recall in this context that
masking only includes or excludes voxels; the value of the mask does not affect the weighting of
voxels beyond whether they are included or not.
3.2.6 – Mask Creation

Mask creation serves two purposes: masks function as a modified rat brain atlas to guide ROI placement, and masks are used with the AIR algorithm to restrict the voxels used to calculate coregistration transformations to only those voxels in and rigidly attached to the brain. Several versions of the mask were created before the final result was created.

The first registration mask was a resampled and resliced version of Toga et al. ’s rat brain atlas. Registration results were improved from unmasked registrations, but there were frequently registration errors in the results. After adjusting registration options, the most persistent errors were rotational. This seems to have occurred because, within the DTBZ PET unilaterally lesioned rat brain image, there is typically only one large activity spike, centred on the unlesioned striatum. This spike is unambiguous in position, and the resliced image can easily be reposition such that the spike is located in the same place in both images. However, because there is only this one activity peak, there is little information in the brain-only masked images to fix the rotational alignment of the images. That is, the resliced image can rotate about its single fixed point – the unlesioned striatum peak – by several degrees and still have a relatively good apparent algorithmic quality of registration. This causes pairs of coregistered PET images to frequently have noticeable rotational misalignments of several degrees. Because the cerebellum is well separated from the striatum, a rotation of up to 5 degrees is sufficient to completely
change the location of the cerebellum between two images, even if the unlesioned striatum is very well registered.

To improve the quality of rotational coregistration, it was realized that additional features in the images being registered would need to be included. These features would ideally be well separated from the striatal peak, and there should be at least two of features, arranged so that all features were not collinear. With three well-separated non-collinear (and, arguably, not forming an equilateral triangle) features appearing prominently, any registration errors of rotation or translation are guaranteed to displace at least one of the features in the resliced image from its corresponding feature in the registration target image.

Luckily, there are conveniently-located glands posterior to the eyes of rats which strongly take up DTBZ. These glands are rigidly fixed relative to the brain, and are spatially separated from each other and the striata. This separation makes them well-suited for anchoring PET to PET image coregistration.

To include the eye glands in the rat brain mask, the brain atlas was fused with edited PET data. To do this, a weakly-lesioned rat DTBZ PET image was selected, and the rat brain atlas was manually registered to the PET image using the RView program (http://rview.colin-studholme.net/). Manual registration consists of typing translations and rotations (though the latter were not deemed necessary) until the two appeared roughly coregistered. The atlas data were then resliced in this new position and saved for later. The weakly-lesioned PET image was then edited to remove, by setting zero, voxels outside of the brain and the activity centres posterior to the eyes. The edited PET data and resliced brain atlas were then combined, and slightly blurred.
Figure 3.5 - Rat brain mask constructed from segmented brain atlas and DTBZ PET data. Atlas has nonzero values throughout brain volume, with high values in the striata and cerebellum where ROIs are placed during PET image analysis. Activity concentrations posterior to eyes are also visible in caudal (left) view. Transaxial view is also shown (right).

The combined mask image shows the atlas-based locations of the striata and cerebellum clearly, allowing it to be used as a guide for ROI placement. Nonzero values are present in the regions over the glands posterior to the eyes, so that they are included in registration calculations, along with the striata. Other regions have voxel values of zero, and are excluded from registration calculations.

It may seem inappropriate or error-prone to register a PET image to the brain atlas data before fusing them to create a mask image for later use. Errors in registration at this stage, which are quite probable given the crude nature of the manually entering translations as a registration method, would seem likely to cause problems in all future applications of the created mask. In practice, this is not an issue, however. The goal of including the activity centres posterior to the eyes in the mask image is not to perfectly align these structures in the mask. Rather, it is only necessary that the nonzero regions of the mask outside the brain cover these structures in PET images to which the mask has been registered in future. Because rat brains are all approximately the same size and shape – which is necessary for an atlas-based ROI-placement method to be valid – the approximate location of the eye glands is also similar between rats. Additionally, the final mask image is blurred, to ensure that the mask extends out beyond the central peaks of the eye gland activity, and that the edge of the mask itself is not reached too soon to make use of the peaks in registration calculations.
3.3 - Separate Image Analysis

3.3.1 - Overview

Image coregistration cannot always be used. It applies only when images are being directly compared and there are reasonable grounds to believe registration will give good and accurate results, such as when the images being compared are repeated scans of the same subject under similar conditions. ROI placement methods that do not rely on coregistration to provide consistency can be used in more experimental situations, such as when a parameter is being varied between multiple subjects without directly comparing experimental results. As well, even when coregistration is used, it is still desirable to place ROIs on the registration target image as accurately as possible. The atlas-guided ROI placement methods discussed below for separate ROI placement can also be used to place ROIs that will be shared between coregistered images.

3.3.2 – Atlas Guidance

The ROI placement method devised in this work uses the Toga et al. rat brain atlas as a guide. Because the AIR registration mask discussed above was created from the same rat brain atlas, it can also be used to guide ROI placement. The extra PET-data-derived features of the mask image do not affect this use of the mask. The method discussed below is summarized in Figure 3.6.

Figure 3.6 - Flow chart for ROI placement

The first step to atlas-guided ROI placement is registration of the atlas to the PET data. It is rare for all relevant PET images in an investigation to be naturally aligned, making this step necessary. The primary method for atlas to PET registration in this work was the RView program’s manual translation and rotation entry, as discussed above. Much effort and attention were directed to consistently performing this step, as it is essential to be able to use the aligned atlas later.
The outline of the rat brain in the mask image was registered to the shape of activity in the PET data that seemed to best match it. RView's threshold for outlining of the mask image was then adjusted so that the striatal peak and cerebellum outlined clearly, and the registration was tweaked to place the atlas outline over the PET. Particular attention was paid to keeping the atlas alignment centred in the medial-lateral direction, and to correctly rotating the atlas about the medial-lateral axis, or equivalently, the rotation in the sagittal plane.

Figure 3.7 - RView program showing mask contour on PET image in three orientations, as seen while registering mask to PET.

Once the brain atlas masks are registered to the PET data, the next step was to place ROIs on the unlesioned striatum, using only the PET data. ROIs placed on the striatum in this work were 3 pixels wide and 5 pixels tall, or 15 pixels total area, which corresponds to 11 mm$^2$ in the image plane. As noted when discussing previous analysis methods (see 3.1.3), there is no difficulty reliably placing ROIs on the unlesioned striatum with DTBZ without atlas-guidance, as it appears distinctly in the image. Rac striata also appear distinctly, although in some cases MP striata may be unclear, in which case the atlas may be employed for guidance.

Having placed the unlesioned striatal ROIs, the aligned mask is then used to provide anatomical context for placing the lesioned side ROIs. The lesioned side striatum can be difficult or impossible to discern, so the appropriate locations for its ROIs is determined by mirroring the placement of the unlesioned ROIs. The rat brain, and its atlas, are symmetrical to
reflections in the midsagittal plane, which allows the lesioned striatum to be located from the unlesioned striatum's location in this way.

Figure 3.8 - ROIs on atlas (left) and PET (right), demonstrating use of atlas to mirror ROI placed on visible unlesioned left striatum to invisible lesioned right striatum.

Lastly, cerebellar ROIs are placed. ROIs placed on the cerebellum in this work were 8 pixels wide and 3 pixels tall, or 24 pixels total area, which corresponds to 18 mm$^2$ in the image plane. There is no reference for placement available other than the location of cerebellum in the atlas for this step. This step is also very important to achieve reliable results, as the cerebellum ROI results are very sensitive to changes of only a single voxel in ROI position in the superior-inferior direction. ROIs are placed at a consistent location for all scans, to minimize this variability.

Figure 3.9 - Five striatal pairs of ROIs (top) and three cerebellar ROIs (bottom) placed on weakly-lesioned rat DTBZ brain PET image.
The ROIs used in this work were rectangular. This was done because programming errors were discovered in the software programs used to place ROIs – ASIPro and MEDx – which caused the area which was averaged to generate ROI TACs to be different from the apparent location of the ROI. Unlike ROIs with curved edges, rectangular ROIs are completely unambiguous with regard to which pixels they cover and the total number of pixels included. This allowed simple tests – generating ROI averages with an image consisting of a single pixel included or excluded form the ROI – to determine whether ROI placement and averaging was being done correctly.

ROIs were placed on five striatal planes and three cerebellar planes. Five planes covered the full extent of the striatal activity peak, although it was often difficult to place ROIs on the outer planes, leading to tests discussed later to compare averaging three and five planes to calculate the striatal-average TACs. Three planes were used on the cerebellum, as this was the extend of the volume in which only cerebellar tissue appeared in the atlas. Including additional planes or using larger ROIs on the cerebellum would have included additional non-cerebellum tissue, due to the irregular outline of that structure.
3.4 – Method Comparisons

3.4.1 – Overview

The quality of ROI placement and coregistration methods was measured by reliability, which is a combination of reproducibility and accuracy. Reproducibility is a measure of how similar are repeated measurements of the same physical and biological quantity. This is quantified by scanning the same animal in similar conditions with the same tracer on separate days, separately analyzing the resulting image and calculating binding potential (BP) (see chapter 4) estimates for both. A figure of merit for reproducibility is the percent change between BP estimates of the same striatum (lesioned or unlesioned) analyzed twice in this manner.

Accuracy is quantified by correlating BP estimates with post mortem autoradiographic binding measurements of the same striatum from the same animal with the same tracer. Autoradiography is treated as a gold standard in this case, which is expected to be linearly related to the BP, allowing the correlation coefficient to be a figure of merit for accuracy.

In addition to testing ROI-placement and image registration methods, the optimal number of PET planes on which to place ROIs for these images was also investigated. In particular, all reproducibility and accuracy tests were done with both three and five planes of striatal ROIs. Three cerebellar planes were used in all analyses.

It is generally expected that repeated scans in similar condition should lead to similar BPs, and that these BPs will correlate well with autoradiography if analysis is done properly. Some variation is expected, however, due to biological changes between scans and the statistical nature of radioactive decay. Over a group of subjects and images, random variation is expected to be averaged out, and average trends to be useful for method comparisons.

Evaluation of ROI placement and registration was done with a set of PET DTBZ rat brain images and autoradiographic binding measurements for the same rats. The rats used for this data set all had multiple PET scans done with DTBZ on separate days, providing independent but similarly-performed estimates of BP. As discussed above, the rats were unilaterally lesioned in
the right striatum with 6-hydroxydopamine (OHDA), which destroys the presynaptic neurons in which the VMAT-2 proteins to which DTBZ binds are found. A range of resulting BPs were produced, allowing meaningful correlations with autoradiography to be done, as well as allowing characterization of reproducibility for a range of BP values.

In total, six rats were used for reproducibility studies, with each rat contributing two DTBZ PET brain scans under similar conditions. An additional four rats were added for correlation with autoradiography, to cover more BP values within the range tested. Hour-long PET scans were reconstructed into 17 frame images, with volumes of 128x128x95 voxels. Striatal ROIs were 3 voxels wide and 5 voxels tall in the transaxial plane, and cerebellar ROIs were 8 voxels wide and 3 voxels tall. Pixels in-plane (perpendicular to the scanner axis) were 0.866 mm in each axis, and slices were 0.796 mm thick.

3.4.2 – Binding Potential Calculation (Brief)

To determine accuracy and reliability figures of merit, BP estimates must be calculated, and this process is briefly summarized: Once ROIs are placed, analysis continues by extracting time activity curves (TACs) from the PET images. The values of the images within the ROIs are averaged for each frame of the image, and the resulting (17 frame, in this work) numbers for each group of ROIs – left striatum, right striatum and cerebellum – are stored with the midpoint times of each frame.

TAC were used as input to kinetic modelling, which will be discussed in detail later (see Chapter 4), using the simplified Logan graphical method. The output of kinetic modelling for TACs of tracers such as DTBZ is a value known as the binding potential (BP), which is a measure of the uptake and binding of the tracer in the tissue within the striatal ROIs, relative to the amounts of tracer present in a reference tissue or blood plasma. Examining BPs under different circumstances allows the results of treatments or interventions to be quantified.
3.4.3 - Autoradiography

Autoradiographic methods used in this work were developed by Strome et al. (2006). Autoradiography is considered to be accurate and suitable for use as a gold standard by which to judge other techniques. The process begins when rats are sacrificed by decapitation, after all necessary PET scans have been performed. Brains were extracted and frozen in isopentane, and stored in freezer at -80°C. Some time later, brains were cut into 16 μm coronal slices, which is equivalent in orientation to PET transverse slices. Slices were made at 5 anterior-to-posterior positions, with 3 redundant slices per position, to representatively sample the whole striatum as seen in PET.

After slicing, separate sets of slices were incubated in 5 nM $^{11}$C-DTBZ solution or 1 μM unlabelled tetrabenazine and 5 nM $^{11}$C-DTBZ combined solution. Incubation with only labelled DTBZ measures the total number of binding sites in the tissue. Incubation with labelled DTBZ and a much higher concentration of unlabelled tracer measures nonspecific binding (see 4.1.1), because the unlabeled tracer fills all available specific binding sites (see 4.1.1) in the tissue.

While brain slice slides dried, activity standard curves were created by serially diluting and pipetting drops of labelled DTBZ solution onto a test slide. Each dilution reduced the concentration, and thus total activity, of labelled DTBZ by a known amount.

After drying, slides and standards were placed against radiosensitive phosphor screens for several hours. After several-hour exposure, nearly all DTBZ has decayed, leaving the total accumulated activity in the phosphor screen proportional to the activity that was taken up by the tissue in the slide that was placed next to it. Screens were then read with a Cyclone storage phosphor system.

Images were analyzed by measuring the image values of the standard drop spots, and creating a fitted relationship between these and the known standard drop concentrations. This standard curve was interpolated between measured data points to quantify the values for the brain slice images.
Circular ROIs were drawn on the brain slice autoradiography images to cover the central region of the striata. When individual brain slices were damaged by the autoradiography process, these slices were ignored. There were sufficient redundant slides that all brain slice regions had at least two redundant slides. ROI averages were taken of the circles, and these averages were converted back to concentration using the standard curve. Nonspecific binding was subtracted from the total binding, to leave just specific binding. The final concentration indicates the number, per volume, of receptors that DTBZ binds to in the tested brain tissue. This concentration of receptors is referred to as $B_{\text{max}}$.

![Figure 3.10 - Autoradiographic $^{11}$C-DTBZ images of rat brain transaxial slices. Circular ROIs are drawn on the striata in the slices shown.](image)

### 3.5 – Results

#### 3.5.1 – Coregistration Visual Inspection

Masked and thresholded AIR registration was very effective at producing images that appeared to be well registered. Both translation and rotation of coregistered images within the
brain is significantly improved, often such that no translation or rotation between images can be seen when comparing them.

Figure 3.11 - Masked AIR coregistration result. Left image is the registration target: the image to which the right image is registered. In the centre image, the right image is resliced to overlie the registration target (left) image. Activity concentration in striatum (red, at intersection of dashed lines) is well registered in resliced and registration target images, but is significantly displaced in the right image prior to reslicing.

### 3.5.2 – Correlation with Autoradiography

Plotting PET BP against autoradiographic binding shows excellent correlation.

Figure 3.12 - 42 ROIs' PET BP estimates plotted against autoradiographic binding measurements for the same striata from 10 rats. PET ROIs were placed with atlas guidance as developed in this work. BPs for both three and five PET planes averaged into each striatal TAC are shown (42 BPs with each of three and five planes averaged), with nearly identical correlation coefficients ($R^2$).
BP values were estimated from TACs placed separately on all striata, using registered mask guidance as discussed earlier in this work. The least-squares best fit lines for PET BP plotted against autoradiographic binding have correlation coefficients $R^2 = 0.947$ for three striatal planes averaged TACs, and $R^2 = 0.941$ for five averaged planes. This is a significant improvement over the $R^2 = 0.64$ reported by Strome et al. (2006) without mask guidance for ROI placement. There is essentially no difference in fit quality between three and five planes averaged TACs. Because placing five planes takes more investigator effort than three planes, it may thus preferable to use three planes for the particular analysis situation investigated in this work. It is also reasonable to assume that similar situations would also have minimal dependence of result quality on number of planes averaged, within a similar anatomical volume.

3.5.3 - Reproducibility

Binding potential reproducibility, defined as the average absolute percent difference between pairs of single-striatum BP estimates are summarized below. These results compare reproducibility with separate ROI placement on each striatum and cerebellum with registered mask guidance, with ROIs placed on one image with mask guidance, and then copied to an image coregistered to the first with the AIR algorithm. Reslicing of the coregistered image was done with a sinc filter which was found not to introduce significant bias when reslicing images of this type. ROIs were placed on three striatal and three cerebellar planes for these tests.

<table>
<thead>
<tr>
<th></th>
<th>N = 12 pairs of BP estimates</th>
<th>Average % difference</th>
<th>st. dev. % of differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Separate ROI Placement</td>
<td>14</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>AIR Coregistration</td>
<td>21</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.1 - Separate and coregistered ROI placement produces differences in BP estimates for each striatum. Average difference and standard deviation of differences are tabulated.

These BP average differences are within half of one standard deviation of each other. The mean difference and standard deviation of difference between individual ROIs' % change between separate and resliced ROI placement are 6% and 24%, with 12 pairs of measurements. The two methods are not statistically different according to the paired t-test.
3.6 - Mask Registration with MNI Register

3.6.1 – Discussion

The MINC software package mentioned above, which includes the minctracc program that was investigated as an alternative to registration with AIR, also includes the register program. Register allows the user to generate spatial transformations of volumetric images, such as PET images and brain atlases. This is done by the user placing pairs of control points on the volumes by clicking on the images. These points are placed such that the user believes they are, spatially, the same location in both images. After four or more such points are placed, the register program calculates a best-fit transformation between the two sets of points. If the chosen control points are placed well, the calculated transformation should coregister the two images.

The register program’s output transformation can be exported and used with the MINC reslice program. Reslice resamples the images it is given as input using the transformation it is given. The MINC package allows transformations to be concatenated and inverted, which allows a large degree of flexibility when processing images and making masks for use with AIR.

Using the flexibility of the MINC package, an alternative method for registering masks to PET images was devised by Katie Dinelle and other members of the research group. A set of PET images was coregistered using MNI register and summed. The rat brain atlas was registered to the summed image, also using MNI register. Each individual PET image is registered to the summed image, and this transformation is inverted and applied to the atlas (registered to the summed image) which creates a transformation to register the atlas to the individual PET image.

This method has produced similar results as manually registering the atlas to the PET scans with RVView. Quantified reproducibility and accuracy numbers are not available however, as this method has not been used on the reproducibility study data set and was not done as part of this work.
Chapter 4: Kinetic Modelling

4.1 – Background

4.1.1 - Overview

Placing ROIs and extracting time activity curves (TAC) is only the first part of the PET brain image analysis procedure. Equally important is the processing of TACs to calculate the biologically relevant parameters that characterize the investigated process for each subject. These values, such as the binding potential (BP) (for reversibly-binding tracers) or uptake rate constant (for irreversibly-binding tracers) provide a quantified measure of the condition of tissues within the voxels or ROIs for which they are calculated. Changes in BP due to intervention or treatment demonstrate the effects of such actions.

Most methods to extract parameters such as the binding potential (BP) from TACs are in a class of methods known as kinetic modelling. These methods assume that the chemical kinetics of the tracer in the subject can be modelled as a series of tracer state compartments. That is, the tracer in a voxel or ROI can be separated into several compartments such as free in blood or plasma, free in interstitial space in tissue, inside cells, or reversibly or irreversibly bound to receptor sites. Mathematical models relate the rate of tracer exchange between compartments to the amount of tracer present in each compartment, and rate constants that are dependent on the tissue-tracer interaction. These models are typically described by differential equations. The input is one or more time activity curves for tissue and / or blood, and the output is the rate constants or functions thereof such as the BP.

Kinetic models also describe how the shape of the time activity curve of tissue depends on the input blood plasma time activity curve, separate reference region TAC for tissue that lacks the specific binding characteristics that are being investigated. For a theoretical tissue that exactly follows a compartmental model, the input time activity curve and the tracer exchange rate constants precisely determine the tissue time activity curve. Real tissue TACs never exactly follow theoretical models, however, because kinetic models are only approximations to the true chemical and physical processes that tracers undergo. As well, PET measurements have
significant statistical noise, which cannot be precisely accounted for in deterministic kinetic models.

4.1.2 - Reversible and Irreversible Systems

Compartmental models with efflux and influx rate that are nonzero for all kinetic compartments are referred to as reversible. If any of the system’s compartments had no nonzero rate constants for transfer out, those compartments would retain tracer they take up, and the entire system would become irreversible (although most models have only one irreversible compartment in practice). Reversible and irreversible systems have different TAC shapes. Reversible systems take up tracer and release it over time back into blood plasma, until equilibrium is reached. Irreversible systems trap some or all tracer in one or more tissue compartments (within the timeframe of a PET scan), causing tracer to be extracted from plasma and tissue concentration to rise (again, if radioactive decay is ignored). As a consequence of these different uptake profiles, different analysis methods are required. In the following, radioactive decay of tracers is ignored; the half-lives of radionuclides used are known and correction factors are applied to data to account for radioactive decay before kinetic modelling begins.

4.1.3 - First Order Systems

Because PET brain scans are performed with very low concentrations of radiolabelled chemical, referred to as tracer concentrations, there is insufficient chemical to alter the system being investigated. Consequently, most tracer kinetic systems can be described adequately by a system of first order ordinary differential equations, with derivatives with respect to time.

First order systems can be characterized by an impulse response, where the total activity in all compartments of the system is the impulse response convolved with the plasma input function time activity curve. This is particularly useful when analyzing PET data, because a PET scan can only measure the total activity of all compartments combined in a voxel; there is no way to directly measure the contribution to the total activity concentration in a voxel from a subset of compartments in the voxel.
4.1.4 - Plasma Input Models

One class of kinetic models involves introducing tracer into the blood, which then circulates through the body and into tissue. The tissue takes up the tracer from the plasma, into the interstitial space, and depending on the specific model, the tracer may transfer back to plasma or exchange with other tissue compartments. Rate constants relate the concentrations or amounts of tracer in each compartment with the rate of transfer to other compartments or to or from plasma.

![Diagram](image)

Figure 4.1 - Example plasma-input compartmental model with three tissue compartments exchanging tracer between themselves, and with the plasma.

Other kinetic models may make different assumptions than the model discussed above. As also discussed earlier, one or more compartments may be irreversible, having a rate constant for transfer of tracer out of the compartment of zero. Additionally, different numbers of compartments may be present, with different arrangements of possible transfers of tracers between them.

In some cases, more complicated models can be simplified by various assumptions to be reasonably approximated by simpler models such as the one discussed above. Whether this is possible or desirable is dependent on the models in question, and in many cases the rate
constants within those models – very large or small rate constants can make one model appear quite similar to another, or make a model behave in usefully distinctive ways.

### 4.1.5 - Reference Regions

Using a kinetic model with a blood plasma input function requires measuring the blood plasma concentration of tracer. Unfortunately, this is not always possible, and even when it is possible, it can be problematic enough to be avoided. For human subjects, measuring blood plasma tracer concentration generally requires placing an arterial sampling line to withdraw blood periodically. Placing a line can be painful and objectionable for patients, and may be a health risk for immune-compromised patients, or may be difficult for experimenters, particularly when patients are mentally unstable. For rat, and especially mouse, studies, placing an arterial line can be quite difficult due to the small size of blood vessels. Additionally, removing significant quantities of blood from a mouse or rat is difficult, due to their small total blood volume.

In some cases, it is possible to eliminate blood plasma sampling by using a PET image-derived TAC as an input function to kinetic modelling instead of a plasma input function (Patlak and Blasberg, 1985; Logan et al. 1996). Image-derived input functions are created the same way as other TACs from images: ROIs are used to average voxel TACs in the appropriate anatomical structures (Patlak and Blasberg, 1985). The region of the image or anatomy that is averaged to create an image-derived input function is referred to as the reference region. By contrast, the tissue being studied is the target tissue.

For a reference region input to be possible, it is necessary that there be an appropriate tissue to act as a reference region for the target tissue that is to be studied. The reference tissue tracer kinetics share one or more compartments with the target tissue, but the target tissue will have additional compartments, absent in the reference region, that model (or cause) the binding that is being investigated in the study. This additional binding in the target tissue is referred to as the specific binding.
The kinetic modelling algorithms used in this work all used a reference region input. The rat striatum was the target tissue, and the cerebellum acted as a reference region.

4.1.6 - DTBZ Rat Striatum Compartmental Model

The biological system that is probed by the tracers used in this work is contained within the rat striatum. DTBZ in particular has a relatively complicated kinetic behaviour in the striatum that is most-fully described by a three tissue compartment model (Koeppe et al., 1996). As well, the PET imaging done in this work does not provide blood plasma input functions, so a reference region compartmental model is necessary. For DTBZ in the cerebellum, a two tissue model is used as the cerebellum reference region lacks the specific binding that is being investigated in the striatum. The compartments of the reference region are also present in the striatum, but the striatum also has an additional compartment that is referred to as the specific binding.

The striatum DTBZ model (figure 4.2) has compartments for tracer free in tissue $C_F$, tracer non-specifically bound, $C_{NS}$, and tracer specifically bound $C_S$. The cerebellum has free tracer $C_F'$ and non-specifically bound $C_{NS'}$ where the ' indicates that a quantity applies to the reference region. Rate constants between these compartments and with plasma are $K_1$ and $K_1'$ for uptake from plasma into the free compartment for target and reference tissues, $k_2$ and $k_2'$ release back into plasma from the free compartment, $k_5$ and $k_5'$ for transfer from free to non-specifically bound compartments, $k_6$ and $k_6'$ for transfer from non-specifically bound to free, and $k_3$ and $k_4$ for transfer from the target tissue free to specifically bound and the reverse.

The particular components of this model were chosen as a compromise between statistical quality of fit of the model to real measured data, and the reproducibility and reliability of such fits (Koeppe et al., 1996). Additional compartments could be added, but this adds additional parameters to be identified, which are often difficult to reliably determine with real data, and can reduce the reliability of estimates of the original parameters. The target tissue component of this model has three tissue compartments, which is generally more compartments than can be distinguished from PET data. In practice, attempting to fit a model of this sort leads to large uncertainties in fitted parameter values, which do not usefully describe tissue properties.
However, various simplifications to the above model can be applied in order to make it practically useful for PET image analysis. These assumptions lead to different algorithms to extract tissue-describing parameters such as the binding potential of the specifically bound compartment with respect to the reference region, and other parameters that will be discussed later.

Figure 4.2 - Rat DTBZ full reference tissue model. Reference tissue has free and non-specifically bound compartments. Target tissue has these, as well as a specifically bound compartment. All compartments bind reversibly.

4.1.7 - Links to Biology and Chemistry

The previously-introduced rate constants for tracer transfer between tissue compartments can be related to biological and chemical concepts that explain their relevance. For the case of ligands binding to receptor sites, the binding and, when reversible, unbinding process is
effectively modelled as a chemical reaction of the ligands, receptors and bound receptor-ligand complexes (Slifstein and Laruelle, 2001). In this context, the concentration of free tracer in tissue is denoted as $F$, the concentration of free receptor sites as $R$, and the concentration of receptor-ligand bound complexes as $B$. In this work, receptor or tracer concentrations are typically in units of (pmol / cc).

When two reagent species are able to freely bind to form a third species, the change in concentration of each with time may be described by the differential equation

$$\frac{dB}{dt} = k_{on}FR - k_{off}B$$

where $k_{on}$ (cc / pmol min) and $k_{off}$ (1 / min) are chemical rate constants. At equilibrium, $dB/dt = 0$ (pmol / cc min), leading to the relation

$$\frac{FR}{B} = \frac{k_{off}}{k_{on}} = K_D$$

where $K_D$ (pmol / cc) is the dissociation constant, a measure of the tendency of the reactive to proceed in reverse. $K_D$ is also the inverse of the affinity of the ligand for the receptor, a measure of their tendency to bind together.

Because $R$ is the concentration of free receptor sites, and $B$ is the concentration of receptors bound with ligand, the total concentration of receptor sites, $B_{max}$ (pmol / cc), is given by

$$B_{max} = R + B$$

However, PET imaging is done at tracer concentrations, which are by definition small enough that the system being investigated is not perturbed. As such, for PET imaging purposes, the total number of receptors, $B_{max}$, is approximately equal to $R$. Given this simplification, the relation above can be rewritten,

$$FB_{max}/B = K_D$$
and furthermore,

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

where BP (unitless) is the binding potential mentioned above.

It should be noted that the binding potential derived here is not necessarily the same BP that would be calculated with PET. PET neurotracer scans are performed on living subjects, however, which have naturally-present, or endogenous, ligand present in the tissue, in vivo. Endogenous ligand binds to receptors sites, just as introduced tracer will, although endogenous ligand and exogenous tracer generally have different affinities for those sites. Endogenous ligand may be present in concentrations greater than tracer levels. This changes the apparent \( B_{\text{max}} \) and \( K_D \). Consequently, the BP calculated here is not the same BP that will be measured with PET, which has fewer receptor sites involved in tracer binding. Conversely, autoradiography is performed with tissue slices in vitro. No endogenous ligand is present, and the true \( B_{\text{max}} \) and \( K_D \) are measured.

Despite this difference in apparent \( B_{\text{max}} \) between PET and autoradiography, it is still useful to correlate the \( B_{\text{max}} \) measured with autoradiography and the binding potential from PET in order to verify the accuracy of PET. This is justified by assuming that the amount of endogenous ligand is similar in all animals involved in the experiments. This endogenous ligand adds an additional constant factor relating the true \( B_{\text{max}} \), from autoradiography, and the PET BP. The quality of correlations is thus unaffected, and correlation with autoradiography \( B_{\text{max}} \) is still a useful means to validate methods that calculate BP.

The relationship given above for transfer of tracer between compartments does not explicitly refer to the concentration of receptor sites, however the chemistry-based description of ligand binding specifically does. For the case of DTBZ specific binding in the striatum, tracer is exchanged between the specifically bound compartment and the free in tissue compartment. These compartments are equivalent to the chemical states of bound ligand and unbound ligand respectively. The \( k_4 \) (1 / min) compartment rate constant, for transfer from specifically bound to free tracer, is equivalent to the chemical \( k_{\text{off}} \); both referring to tracer transferring from being
bound to receptors to its unbound state, with the total rate of transfer being proportional to the amount of bound ligand present.

A similar relationship exists between $k_{on}$ and $k_3$, where $k_3 (1 / \text{min})$ is the rate constant for transfer from the free to specifically bound compartments. Similarly to $k_4$ and $k_{off}$, this rate is equal to the rate of binding of ligand to receptors. Unlike $k_4$ and $k_{off}$, however, the concentration of unbound tracer is not the only factor determining the rate $k_3$. Rather, as seen in the chemical description, both the concentration of unbound tracer and the concentration of binding sites affects the total binding rate. This can be reconciled by expressing $k_3$ in terms of $k_{on}$ and $B_{max}$,

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

That is, the rate constant for transfer to the specifically bound compartment is the product of the single-receptor constant and the number of receptors. Each receptor contributes to the total binding rate constant of the tissue.

Additionally, by rearranging the above equations, the BP may be expressed in terms of these same rate constants,

$$BP = B_{max}/K_D = B_{max}k_{on}/k_{off} = k_3/k_4$$

This finally links BP explicitly to all rate constants and the number of receptor sites. Note that "BP" here is the binding potential of the target tissue specifically bound compartment with respect to the target tissue free tracer compartment. This is written as $BP_T$ by Innis et al (Innis et al., 2007), to distinguish between alternative definitions of BP, which may be written relative to the non-displaceable compartment: $BP_{ND}$, which is the combination of free and nonspecifically bound, or the tracer in plasma: $BP_p$
4.2 – Algorithms

4.2.1 - Plasma Input Logan Graphical Method

Many compartmental modelling analysis methods require assumptions about the number of compartments that describe tracer kinetics. The Logan graphical methods (Logan et al., 1990) are unusual in this respect; they require no assumptions other than that there is some (unknown) number of reversible tissue compartments exchanging tracer with each other and with the blood plasma, and that equilibrium between compartments is reached during the course of the study. The exchange of tracer between tissue compartments is described by a set of rate constants, such as that discussed for the DTBZ rat striatum compartmental model. These constants are written as a matrix $K$, which has units of inverse time, and used in the differential equation,

$$\frac{dA}{dt} = KA + K_1C_p$$

where $A$ is a column vector of compartment activities and here $K_1$ is a column vector with the scalar rate constant $K_1$ discussed above in the row corresponding to the activity in the compartment into which tracer is transferred from plasma (the free tracer in tissue compartment in the case of DTBZ). The total tissue activity in this formulation is the sum of the rows of $A$, or $A$ multiplied by a row vector of 1's.

![Diagram](image)

Figure 4.3 - Example two tissue compartment reversible plasma input kinetic model

Integrating the above and rearranging to isolate the integral of $A$ gives
\[ \int_0^T A(t) dt = -K^{-1} K_1 \int_0^T C_p(t) dt + K^{-1} A(t) \]

where \( K^{-1} \) is the matrix inverse of \( K \). Multiplying by a row vector of 1’s, \( U \), gives

\[ \int_0^T A_T(t) dt = -U K^{-1} K_1 \int_0^T C_p(t) dt + U K^{-1} A(t) \]

where \( A_T(t) = U A_T(t) \) is the total tissue activity. Alternatively, if the volume of blood plasma in the tissue is significant, the plasma volume \( V_p \) may be added explicitly,

\[ \int_0^T A_T(t) dt = -(U K^{-1} K_1 + V_p) \int_0^T C_p(t) dt + U K^{-1} A(t) \]

Dividing by \( A_T(t) \) gives

\[ \int_0^T A_T(t) dt / A_T(t) = -(U K^{-1} K_1 + V_p) \int_0^T C_p(t) dt / A_T(t) + U K^{-1} A(t) / A_T(t) \]

After the fast components of the tracer exchange between compartments have decayed, the activities in all compartments have equilibrated, and there is no net exchange in activity if the plasma concentration remains constant. This occurs after some time \( t^* \), after which,

\[ 0 = dA/dt = KA + K_1 C_p \]

\[ A = K^{-1} K_1 C_p \]

As well, in the above equation, the rightmost term \( U K^{-1} A(t) / A_T(t) \) is a constant. This leaves a simple linear relationship of the form
\[ Y = M X + B \]
\[ Y = \int_{0}^{T} A_T(t) \, dt / A_T(t) \]
\[ X = \int_{0}^{T} C_p(t) \, dt / A_T(t) \]

where the slope \( M = -(U K_1 + V_p) \) is the distribution volume (DV) of this tissue with respect to plasma, and \( B \) is the constant term. The DV is the volume of plasma that would have the same total amount of tracer as unit volume of tissue, with units of volume per volume, but treated as unitless in practice. For a one tissue compartment model, the DV is equal to \( K_1 / k_2 \):

\[
dA_T/dt = K_1 C_p - k_2 A_T \\
A_T = K_1 \int_{0}^{T} C_p(t) \, dt - k_2 \int_{0}^{T} A_T(t) \, dt \\
\int_{0}^{T} A_T(t) \, dt / A_T(t) = (K_1/k_2) \int_{0}^{T} C_p(t) \, dt / A_T(t) - 1 / k_2
\]

Similarly, for a two tissue model, DV is equal to \( (K_1/k_2)(1 + k_3/k_4) \):

\[
A_{ND} = K_1 C_p - (k_2 + k_3)A_{ND} + k_4 A_S \\
A_S = k_3 A_{ND} - k_4 A_S \\
A_S = (1/k_4)(k_3 A_{ND} - A_S) \\
A_{ND} = K_1 C_p - (k_2 + k_3)A_{ND} + k_3 A_{ND} - A_S \\
A_T = K_1 C_p - k_2 A_{ND} \\
A_T = K_1 C_p - k_2 A_T(A_{ND}/A_T)
\]

at equilibrium, \( dC_S/dt = 0 \)
\[
C_S = (k_3/k_4)C_{ND} \\
C_T = (1 + k_3/k_4)C_{ND} \\
A_T = (1 + k_3/k_4)A_{ND}
\]
\[ A_T = K_1 C_p - \left( \frac{k_2}{1 + k_3/k_4} \right) A_T \]

which is of the same form as above for one compartment, leading to

\[
\int_0^T A_T(t) \frac{dt}{A_T(t)} = \left( 1 + \frac{k_3/k_4}{K_1/k_2} \right) \int_0^T C_p(t) \frac{dt}{A_T(t)} - \left( 1 + \frac{k_3/k_4}{k_2} \right)
\]

4.2.2 - Reference Input Logan Graphical Method

The reference input Logan method was originally derived by taking the ratio of the separate distribution volumes (DV) of a reference region (DV_R) and target a region (DV_T) (Logan et al., 1994, Logan et al. 1996). The reference region is assumed to have one compartment, so that

\[ DV_R = \left( K_1'/k_2' \right) \]

while the target tissue may have any number of compartments. For the one tissue model, DV_T is of the same form as DV_R, except with K_1 and k_2 replacing K_1' and k_2', respectively. For a two compartment target tissue model,

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

and other similar expressions could be derived for more complicated models.
Figure 4.4 - Example two target tissue and one reference tissue compartment model. The "BOUND" compartment is specific to the target tissue, and is absent from the reference tissue, and causes differences between the target and reference TACs.

It is assumed that the target $K_1$ and reference $K_1'$ are related by $K_1 = R_1 K_1'$, with the same ratio between the target $k_2$ and reference $k_2'$, $k_2 = R_1 k_2'$. As such, $K_1/K_1' = k_2/k_2'$ and $K_1/k_2 = K_1'/{k_2'}$. Consequently, by dividing these DVs, one gets their ratio, referred to as the distribution volume ratio (DVR), which happens to be equal to $1 + k_3/k_3'$ in the two target tissue case, or $1 + BP$ in general.

By combining the formula for the tissue $DV_T$ and reference $DV_R$, a single relation that does not contain the blood plasma concentration can be obtained. Mathematically, this involves treating the reference tissue as an input function to the target tissue, though no such direct physical connection is presumed to exist, as the two brain structures are spatially separated.

\[
\int_0^T A_T(t) \, dt / A_T(t) = DV_T \int_0^T C_p(t) \, dt / A_T(t) + \text{int}
\]

\[
\int_0^T A_T(t) \, dt / A_R(t) = DV_R \int_0^T C_p(t) \, dt / A_R(t) + 1 / k_2'
\]

where \( \text{int} \) is an intercept term. Rearranging to isolate the integral of $C_p(t)$.
\[ \int_0^T C_p(t) dt = (1/DV_R)(\int_0^T A_R(t) dt - A_R(t)/k_2) \]

and substituting for the integral of \( C_p(t) \) in the equation for \( DV_T \),

\[ \int_0^T C_T(t) dt / A_T(t) = DV_T / DV_R \left( \int_0^T A_R(t) dt - A_R(t)/k_2' \right) / A_T(t) + \text{int} \]

\[ \int_0^T A_T(t) dt / A_T(t) = DV_R \left( \int_0^T A_R(t) dt - A_R(t)/k_2' \right) / A_T(t) + \text{int} \]

As well, the \( A_T \) and \( A_R \) in these expressions may be replaced with \( C_T \) and \( C_R \) respectively, without changing the resulting \( DVR \):

\[ \int_0^T C_T(t) dt / C_T(t) = DV_R \left( \int_0^T C_R(t) dt - C_R(t)/k_2' \right) / C_T(t) + \text{int} \]

In this work, this relation is referred to as the standard Logan (graphical) algorithm or method. The reference region \( k_2' \) in this expression is not derived from the data itself, so is generally supplied from population values for human. For rats, the value of \( k_2' \) is sufficiently large that the \( C_R(t)/k_2' \) term may be omitted from the entire expression without significantly affecting the results, as discussed in section 4.6, below.

\[ \int_0^T C_T(t) dt / C_T(t) = DV_R \left( \int_0^T C_R(t) dt \right) / C_T(t) + \text{int} \]

This relation is referred to as the simplified Logan method.

These methods are effective and widely used, but have drawbacks in that they are biased by noise. Noisier TACs will on average give a lower DVR estimate, due to correlation in the noise between the left and right hand sides of the equation (Slifstein and Laruelle, 2000).
4.2.3 - Full Reference Tissue Model

The full DTBZ rat striatum reversible compartmental model described above (section 4.1.6) is in practice not used without simplification. The non-specifically bound and free compartments equilibrate very quickly, and are effectively indistinguishable in PET TACs. By grouping these compartments together (Frey et al., 1985; Slifstein and Laruelle, 2001) into a non-displaceable compartment, the so-called full reference tissue model is derived. The reference tissue has a single non-displaceable compartment, containing concentration $C_R = C_{ND}'$, and the target tissue has both the non-displaceable and specifically bound compartments, $C_{ND}$ and $C_S$, respectively.

The relevant rate equations are,

$$C_{ND} = C_F + C_{NS}$$

$$C_R = C_{ND}' = C_F' + C_{NS}'$$

$$C_{ND} = K_1 C_p - (k_2 + k_3) C_{ND} + k_4 C_S$$

Figure 4.5 - DTBZ full reference tissue model with free-in-tissue and non-specifically-bound compartments combined into non-displaceable compartment in target and reference tissues.
\[
C_S = k_3C_{ND} - k_4C_S
\]
\[
C_R = K_1'C_D - k_2'C_R
\]
\[
C_T = C_{ND} + C_S
\]
\[
C_T = R_1C_R + k_2C_R - k_2/(1+BP)C_T
\]

and the solution is (Slifstein and Laruelle, 2001),

\[
C_T = R_1C_R + [B_1 e^{-a_1t} e^{a_1t} + B_2 e^{-a_2t}] \otimes C_R
\]

where \(C_T\) and \(C_{NS}\) are the free and non-displaceable compartments in target tissue, \(C_{F}'\) and \(C_{NS}'\) are the free and non-displaceable compartments in reference tissue, \(C_{ND}'\) and \(C_R\) are the reference tissue concentration, \(C_{ND}\) is the target tissue non-displaceable compartment, \(C_S\) is the target tissue specifically bound compartment, \(CT\) is the total target tissue concentration, \(K_1\) and \(K_1'\) are the target and reference tissue rate constants for uptake from plasma into the non-displaceable compartments, \(k_2\) and \(k_2'\) are the target and reference tissue rate constants for release to plasma from non-displaceable compartments, \(R_1 = K_1/K_1' = k_2/k_2'\) is the delivery ratio for tracer to target with respect to reference tissue, \(B_1, B_2, a_1\) and \(a_2\) are constants to be determined by fitting the observed target tissue and reference tissue TACs to the model indicated by the equations above, and \(\otimes\) indicates convolution. The final model equation in the above is found by solving the system given by the preceding differential equations.

The algorithm to determine the \(B\) and \(a\) in the above model is a nonlinear search. This is an error prone and time consuming type of calculation, particularly for summed exponentials, which are particularly difficult to distinguish from each other. As well, because this model attempts to fit four separate parameters, the uncertainty in each of the fitted results is quite high.
4.2.4 - Multilinear Reference Tissue Model

As an alternative solution to the equation derived for the full reference tissue model, the equation itself can be integrated. This results in the linearized reference tissue model (Ichise et al., 2003),

\[ C_T(T) = R_1C_R(T) + k_2 \int_0^T C_R(t)dt - k_2/(1+BP_{ND}) \int_0^T C_T(t)dt \]

where \( T \) is one of the PET image frame times. This model is used to calculate \( R_1 \), \( k_2 \) and \( BP_{ND} \), where again, \( BP_{ND} \) (hereafter just \( BP \)) is the binding potential of the specifically bound compartment with respect to the non-displaceable compartment, which is the combination of the free and the nonspecifically bound compartments. The calculation proceeds by integrating \( C_R \) and \( C_T \) from the start time of the PET scans, labelled time 0, to the time of the last frame. Each frame in the scan produces a separate version of the above equation. Placing these versions into a matrix, so that the columns comprise each term in the equation for each frame time, produces an overdetermined multilinear problem. The matrix columns contain the values of

\[ C_R(T), \int_0^T C_R(t)dt, \text{ and } \int_0^T C_T(t)dt \]

for times \( T \) from the first to final frame. An additional column vector contains \( C_T(T) \) for the same times \( T \). The solution to the problem is the set of \( R_1 \), \( k_2 \) and \( k_2/(1+BP) \) that gives the least squares best fit for the available data. This is a generic matrix algebra problem that is easily and quickly solved by many computer algebra systems.

4.2.5 - Aside: Two Tissue Logan Method

It can be illuminating to note that, in the two target tissue compartment case with reference tissue input, the multilinear equation can be rearranged in the following manner:

\[ C_T(T) / C_T(T) = R_1C_R(T) / C_T(T) + k_2 \int_0^T C_R(t)dt / C_T(T) - k_2/(1+BP) \int_0^T C_T(t)dt / C_T(T) \]

\[ (1+BP) / k_2 = ((1+BP)R_1 / k_2)C_R(T) / C_T(T) + (1+BP) \int_0^T C_R(t)dt / C_T(T) - \int_0^T C_T(t)dt / C_T(T) \]
\[
\int_0^T C_T(t) dt / C_T(T) = (1 + BP) \int_0^T C_R(t) dt / C_T(T) + ((1 + BP) R_1 / k_2) C_R(T) / C_T(T) - (1 + BP) / k_2
\]

\[
\int_0^T C_T(t) dt / C_T(T) = DVR(\int_0^T C_R(t) dt + C_R(T) / k_2 pop / C_T(T) + DVR(C_R(T) / C_T(T))(1 / k_2' - 1 / k_2' pop) - DVR / k_2
\]

This is the same form as the standard Logan method in the two target tissue case (although again, the Logan method does not assume any particular arrangement of kinetic compartments).

### 4.2.6 - Simplified Reference Tissue Model

An additional simplification to the DTBZ-like compartmental model is to assume that the free, nonspecifically bound and specifically bound compartments all equilibrate quickly. In this case, the target tissue TAC acts as though it has only a single compartment. This model works for tracers such as Rac, but other tracers, including MP and DTBZ, require additional compartments to explain their TAC shapes.

The simplified reference tissue model appears mathematically as a single tissue compartment reference tissue, with the solution given below (Lammertsma and Hume, 1996),
\[ C_R = K_1'C_p - k_2'C_R \]

\[ C_T = R_1C_p - k_{2\text{app}}C_T \]

\[ C_T = R_1C_R + k_2(1 - R_1/(1+BP))C_R \otimes e^{-k_{2\text{app}}t} \]

where \( k_{2\text{app}} \) is, for a true one-tissue compartment system, equal to the \( k_2 \) given above: transfer from the first tissue compartment to back to plasma. In a system that actually has two tissue compartments that are quickly equilibrating, \( k_{2\text{app}} \) is the apparent \( k_2 \), which is mathematically equal to \( k_2/(1+BP) \). Knowing \( k_2 \) and \( k_{2\text{app}} \), BP may be calculated in this case.

The equation for the simplified reference tissue model is similar in form to the full reference tissue model equation, and shares some of its drawbacks. The one of the parameters is nonlinear, making finding an optimal solution with standard optimisation methods difficult and error prone.

**4.2.7 Alternative Simplified Reference Tissue Model**

The nonlinearity of the simplified reference tissue model is entirely due to the parameter \( k_{2\text{app}} \) in the exponent, unlike the full reference tissue model which has two nonlinear parameters. A shortcut or simplifications developed by Gunn et al. (Gunn et al., 1997) uses this fact to make the simplified reference tissue method significantly faster and more reliable. This is done by treating the nonlinear parameter differently than the linear parameters. Rather than simultaneously solving the system for all three parameters, the nonlinear parameter is treated as a constant, and the linear parameters are solved using the simpler linear matrix mathematics as described for the linearized reference tissue model. The system is solved repeatedly, with a set of \( k_{2\text{app}} \) values spread over the limited realistic physical (or biological) range of values it might take.

To find the overall best solution, the solutions for each assumed \( k_{2\text{app}} \) are ranked by the unexplained regression errors in their least-squares best fit. The best fit is chosen, and used as the final nearly-optimal solution to the nonlinear problem. In practice, the quality of the other
resulting parameter values — BP and R₁ — are not very sensitive to small changes in the k₂app parameter. As such, this method works well when tracer kinetics are adequately described by a one target tissue model.

4.2.8 - Expectation Maximization Impulse Response Method

Holden (Holden, 2007) noted that the time activity curve of a reversible tissue can be described as the input curve, from plasma or a reference tissue activity curve, convolved with an impulse response.

\[ C_T = C_p \otimes I_{TP} \]
\[ C_R = C_p \otimes I_{RP} \]

where \( I_{TP} \) is the impulse response of target tissue with respect to plasma, and \( I_{RP} \) is the impulse response of reference tissue with respect to plasma. As well, the target tissue may be expressed as a convolution of the reference tissue and an impulse response with respect to the reference tissue.

\[ C_T = C_R \otimes I_{TR} \]

where \( I_{TR} \) is the impulse response of target tissue with respect to reference tissue. This may be expanded by replacing \( C_R \) with its expression in terms of \( C_p \)

\[ C_T = C_p \otimes I_{RP} \otimes I_{TR} \]

Consequently, one may conclude that

\[ I_{TP} = I_{RP} \otimes I_{TR} \]

Additionally, for a reversible system,

\[ \int_0^T C_T(t)dt = D V_T \int_0^T C_p(t)dt + D C_T(T) \]

where D is some constant. As well,
\[
\int_{0}^{\infty} C_T(t) dt = \int_{0}^{\infty} C_p \otimes I_{TP} dt
\]
\[
\int_{-\infty}^{\infty} (f \otimes g) dt = \int_{-\infty}^{\infty} f dt \int_{-\infty}^{\infty} g dt
\]

\[C_T(t < 0) = C_p(t < 0) = I_{TP}(t < 0) = 0\]

So,

\[DV_T \int_{0}^{\infty} C_p dt + D C_T(\infty) = \int_{0}^{\infty} C_T dt = \int_{0}^{\infty} C_p \int_{0}^{\infty} I_{TP} dt\]

and thus

\[DV_T = \int_{0}^{\infty} I_{TP} dt\]

and also,

\[\int_{0}^{\infty} I_{TP} dt = \int_{0}^{\infty} I_{TR} \otimes I_{RP} dt = \int_{0}^{\infty} I_{TR} dt \int_{0}^{\infty} I_{RP} dt = DVR \int_{0}^{\infty} I_{TR} dt\]

\[\int_{0}^{\infty} I_{TR} dt = DV_T / DVR = DVR\]

As such, knowing the impulse response of target tissue with respect to reference tissue allows the DVR, and thus BP to be calculated.

Recovering the impulse response, IRF(t), requires deconvolving the target tissue time activity curve C_T(t) with reference time activity curve C_R(t). Holden employs a deconvolution algorithm that is similar in concept to iterative PET reconstruction. An initial guess IRF is chosen, and then convolved with the known C_R. The predicted C_T is compared with the measured C_T, and correction factors for the IRF are computed. After sufficient iterations, the IRF estimate generally produces good agreement between the predicted and measured C_T, and the integral of the IRF provides an estimate of the BP. The correction factors are chosen to give the impulse response with the maximum likelihood given the measured tissue TACs. The method is thus referred to as expectation maximization impulse response (EMIR).
Similar to the Logan graphical methods, EMIR does not inherently impose any assumptions on the form of the impulse response or the compartmental nature of the system it describes, other than that there is an impulse response with finite area. Finite area implies that the system being probed is reversible. In practice however, it can be beneficial to impose a single decaying exponential shape on the trailing ends of the IRF. Similar to the Logan assumption that, after some time $t^*$, the various tissue compartments have equilibrated, it is often the case that the IRF follows a single exponential after a similar time.

In order to be able to compute correction factors, the target tissue TAC must be available for the corresponding timepoints of the impulse response. Because of this limitation, it is not mathematically possible to calculate correction factors for the impulse response at timepoints later than the full duration of the measured tissue time activity curve; the IRF is limited to this duration. The majority of the area of the IRF generally falls within the range – if it did not, the duration of the PET scan would be insufficient to usefully characterize the response of the tissue to the tracer. However in some cases, the IRF does not come sufficiently close to zero by the end of the calculated range, and some additional IRF area is contained outside the area that is calculated. This additional area can have a noticeable contribution to the DVR (and thus BP) and needs to be accounted for. This is done by fitting a single decaying exponential curve to the latter portion of the IRF, after the time $t^*$ at which it takes on this shape because all target tissue compartments have equilibrated. A decaying exponential’s area can be easily extrapolated to infinite time – as required by the formal definition of the DVR in terms of the IRF area – and used to correct the DVR estimate.

4.3 - Modified Graphical Method

4.3.1 - Derivation

While investigating the above algorithms, it was realized that a modification of the Logan graphical methods was possible that have some theoretical advantages over the standard Logan method. As discussed above, the standard Logan method has the form,
\[
\int_0^T C_T(t)dt / C_T(T) = DVR \left( \int_0^T C_R(t)dt - C_R(T)/k_2' \right) / C_T(T) + \text{int}
\]

This equation is of the form

\[Y = MX + B\]

\[X = \int_0^T C_T(t)dt / C_T(T)\]

\[Y = \left( \int_0^T C_R(t)dt - C_R(T)/k_2' \right) / C_T(T)\]

and the slope \(M = DVR\) is evaluated by calculated \(X\) and \(Y\) for several times after time \(t^*\). \(t^*\) is defined by the time at which the reversible compartment has equilibrated. This means that the non-displaceable compartment \(C_{ND}\) and the specifically bound compartment \(C_S\) concentrations are at a constant ratio. Because the reference concentration \(C_R\) is essentially a non-displaceable compartment itself, this implies that there is also a constant ratio between the reference, target non-displaceable and target specific compartments, and thus between the reference and total target compartments.

\[C_T(t) / C_R(t) = \text{const. } t > t^*\]

Accordingly, one can multiply both sides of the Logan equation by \(C_T(t) / C_R(t)\) without affecting the slope or the validity of the assumptions about when the equation applies.

\[\int_0^T C_T(t)dt / C_T(T) = DVR \left( \int_0^T C_R(t)dt - C_R(T)/k_2' \right) / C_R(T) + \text{int'}\]

\[\int_0^T C_T(t)dt / C_R(T) = DVR \int_0^T C_R(t)dt / C_R(T) - DVR / k_2' + \text{int'}\]

where \(\text{int'}\) is a new intercept that is equal to the old intercept \(\text{int}\) multiplied by \(C_T(T) / C_R(T)\).
If a particular compartmental model is chosen, such as the two tissue compartments, $int$ and $int'$ may be specified, as discussed above in an aside:

$$\int_0^T C_T(t) dt / C_T(T) = DVR \int_0^T C_R(t) dt / C_T(T) + (DVR R_1 / k_2) C_R(T) / C_T(T) - DVR / k_2$$

Multiplying by $C_T(T) / C_R(T)$,

$$\int_0^T C_T(t) dt / C_R(T) = DVR \int_0^T C_R(t) dt / C_R(T) + (DVR R_1 / k_2) - DVR C_T(T) / (C_R(T) k_2)$$

where again, $k_2' = k_2 / R_1$, and the form of $int'$ becomes clear for the two target tissue case. From this, the rightmost term may be pulled into the term immediately to the right of the equality,

$$\int_0^T C_T(t) dt / C_R(T) = DVR(\int_0^T C_R(t) dt - C_T(T) / k_2) / C_R(T) + (DVR R_1 / k_2)$$

This recreates the form of the standard Logan graphical reference input relation, except that $C_R(T)$ is substituted for $C_T(T)$ in the denominators, and $C_T(T) / k_2$ is substituted for $C_R(T) / k_2'$ in the first right hand side term. The $C_T(T) / k_2$ would act similarly to its equivalent in the standard Logan method, causing the relationship to become linear faster than it otherwise would, but this term cannot be used. This is because the target tissue $k_2$ varies from subject to subject and voxel to voxel, and depends on lesion severity, so a single population value, or even a single value for a single animal, cannot be used. Consequently the modified graphical method omits this term, and is only intended for use in cases where the extra linearizing terms are not necessary.

$$\int_0^T C_T(t) dt / C_R(T) = DVR(\int_0^T C_R(t) dt) / C_R(T) + (DVR R_1 / k_2)$$
4.4 - Parametric Imaging

4.4.1 - Overview

Parametric imaging is an alternative method to PET image analysis in which individual voxel time activity curves have parameters, such as BP, generated for them, rather than first averaging multiple voxels' TACs with ROIs. The calculated parameters are stored in an image volume similar to the PET image itself, though having only a single frame of data. The parameters can be viewed and averaged with ROIs, similar to PET images. When coregistered, parametric images from before and after interventions or treatments can be examined for statistically significant changes, without first having to place ROIs.

Algorithms to calculate parameters like the BP do not work on single-voxel TACs as well as they do for ROI-averaged TACs, however. The primary cause of this problem is noise; single voxel TACs contain information from a much smaller number (N) of recorded photon interactions, and the resulting statistical noise, proportional to $1/\sqrt{N}$, is much larger. As well, in reconstructed images, enough correlation is introduced between adjacent voxels' noise that a Poisson distribution is not accurate for nearby voxels.

Noisy voxel TACs mean that repeated measurements will produce different results. This makes it difficult to be sure that changes between two images are due to actual physiological or biochemical changes in the subject, and not due to random chance altering voxels. Even if restrictions are imposed that adjacent voxels must show similar changes, with a large number of noisy voxels, some coincidentally similar changes in close proximity are expected. That said, statistical analysis packages such as SPM (Wellcome) do exist which can deal with voxel TAC noise and extract only significant changes between images.

Some algorithms are unstable in the presence of noise. There are relatively few frames in a dynamic PET image, and those frames are unevenly distributed in time. At earlier times, when tracer kinetics are changing more rapidly and before compartments have equilibrated, frames are closely spaced and short in duration – as little as 30 seconds in this work. At later times, tracer kinetics have stabilized, and frames are more than five minutes in duration. If a single long-
duration frame is affected by noise, its value can be increased significantly. Because this frame is long, the total activity implied by a long, high valued frame can completely throw off the results of the time-integrals of activity that are commonly used in compartmental modelling. This can cause completely unrealistic, or simply wrong, parameter estimates in some voxels. The presence of these bad voxels can make useful analysis of images difficult or impossible, depending on the ratio of reasonable to obviously bad voxels. Gunn's modification of the simplified referenced tissue model and Holden's EMIR were found to be prone to this problem, with large numbers of completely unreasonable parameter estimates often appearing in voxels throughout the brain volume.

More troubling, however is noise-induced bias. Some analysis methods have a noise-dependent bias in output BP, making them unsuitable for use with noisy TACs such as those analyzed in parametric imaging. The Logan graphical method has been shown to have a theoretical negative bias in BP (Slifstein and Laruelle, 2000) due to correlation between the x and y axes of the plotted data (integral of $C_R$ divided by $C_T$ and integral of $C_T$ divided by $C_T$, respectively). That is, more noisy data on average gives lower BP estimates with the Logan graphical methods. This is particularly troubling, given the Logan methods' wide use. Other methods are also observed to have biases as well; the linearized full reference tissue model seems to have a positive bias in BP with increased noise, as seen by comparing the ROI TAC BP estimate with an average of the parametric image single voxel BP estimates with the same ROI used to create the ROI TAC.

Bias can also be introduced when attempting to resolve other problems with parametric imaging. One method that was investigated to improve the quality of parametric image generation, particularly when many obviously bad voxels were appearing in parametric images, was to ignore those bad voxels. Various schemes could be employed to replace these bad voxels, such as averaging the neighbouring values, or the bad voxels could be ignored entirely when analyzing images. While this method did improve apparent image quality, it was found to introduce significant bias of its own in the resulting average calculated BP. In the case of the Gunn method, the bad voxels were those which had an increase in the value of the TAC for late timepoints. By preferentially removing voxels which had above average values at late times, the effect was to bias the overall average TAC to have lower than its true average value for those
late times. This negatively biased the calculated average BP. Similar bad voxels were observed with EMIR, and similarly increased voxel values near the end of the target tissue TAC seemed to be present.

4.4.2 - Two-Step Parametric Modelling

Some of the kinetic modelling algorithms discussed above can be modified to improve their robustness to TAC noise when doing parametric imaging. This is done using a two-step modelling procedure, with an initial step using a less-noisy ROI TAC input, and the subsequent full parametric image generation step using the part of the results of the initial step to constrain its results to more reasonable ranges. The initial step fits the full three-parameter model (Gunn’s SRTM or linearized FRTM), which determines \( k_2 = R_i k_2' \) based on the ROI TAC. The ROI TAC used could be from one or both striata; in this work it was found to make little difference in the final results whether the unlesioned, lesioned or average was used. The ROI \( k_2' \) is taken as a constant, and a second step where a simplified two parameter model is fitted to the individual voxel takes is done. This assumption is reasonable in theory, as the same reference region is used for all individual voxel TACs, and the reference region \( k_2' \) should not change if the same reference region is used. Target tissue \( k_2 \) and \( R_1 \) values do change from voxel to voxel, however, along with the BP.

For the linearized full reference tissue model, the ROI TAC equation is

\[
C_T^{\text{avg}}(T) = R_1^{\text{avg}} C_R(T) + k_2^{\text{avg}} \int_0^T C_R(t) dt - k_2^{\text{avg}}/(1 + B_P^{\text{avg}}) \int_0^T C_T^{\text{avg}}(t) dt
\]

where the superscript \( \text{avg} \) now indicates ROI TAC averaged values. The first two of the three averaged-TAC parameters here, \( R_1^{\text{avg}} \), \( k_2^{\text{avg}} \) and \( k_2^{\text{avg}} / (1 + B_P^{\text{avg}}) \), may be rearranged to extract \( k_2^{\text{avg}} / R_1^{\text{avg}} = k_2^{\text{avg}} \). If this \( k_2^{\text{avg}} \) is taken as a constant, the equation can be rewritten for a single voxel TAC with fewer parameters (Wu and Carson, 2002).

\[
C_T(T) = R_1 C_R(T) + k_2 \int_0^T C_R(t) dt - k_2(1 + B_P) \int_0^T C_T(t) dt
\]
\[ C_T(T) = R_1 C_R(T) + R_1 k_{2}^{avg} \int_{0}^{T} C_R(t) dt - k_2/(1+BP) \int_{0}^{T} C_T(t) dt \]

\[ C_T(T) = R_1 C_R(T) + k_{2}^{avg} \int_{0}^{T} C_R(t) dt - k_2/(1+BP) \int_{0}^{T} C_T(t) dt \]

\[ C_T(T) = R_1 k_{2}^{avg} \frac{C_R(T)/k_{2}^{avg} + fC_R(t) dt}{(1+BP)k_{2}^{avg}} \]

\[ C_T(T) = k_2( C_R(T)/k_{2}^{avg} + \int_{0}^{T} C_R(t) dt ) - k_2/(1+BP) \int_{0}^{T} C_T(t) dt \]

where the solution is the two parameters \( k_2 \) and \( k_2/(1+BP) \), from which the BP may be easily calculated for each voxel.

Similarly, for the modified simplified reference tissue model, the ROI TAC equation is

\[ C_T^{avg} = R_1^{avg} C_R + k_{2}^{avg}(1 - R_1^{avg}/(1+BP^{avg}))C_R \otimes e^{k_{2}^{avg}t} \]

The three averaged-TAC parameters here, \( R_1^{avg}, k_{2}^{avg}(1 - R_1^{avg}/(1+BP^{avg})) \), \( k_{2}^{avg}/(1+BP^{avg}) = k_{2}\text{app} \), may be combined to extract \( k_{2}^{avg}/R_1^{avg} = k_{2}^{avg} \).

\[
\text{term 1} = R_1^{avg} \\
\text{term 3} = k_{2}^{avg}/(1+BP^{avg}) \\
\text{term 2} = k_{2}^{avg}(1 - R_1^{avg}/(1+BP^{avg})) \\
= k_{2}^{avg} - k_{2}^{avg} R_1^{avg}/(1+BP^{avg}) \\
= k_{2}^{avg} R_1^{avg} - k_{2}^{avg} R_1^{avg}^2/(1+BP^{avg}) \\
= R_1^{avg} (k_{2}^{avg} - k_{2}^{avg}/(1+BP^{avg})) \\
= \text{term 2} = \text{term 1}(k_{2}^{avg} - \text{term 3}) \\
k_{2}^{avg} = \text{term 3} + \text{term 2}/\text{term 1}
\]

If this \( k_{2}^{avg} \) is taken as a constant, the equation can be rewritten for a single voxel TAC with fewer parameters.
After using the original Gunn method to find the optimal $k_2'$ for an ROI TAC, the $k_{2'\text{avg}}$ is fixed. A set of $k_{2\text{app}}$ values are chosen, and with each taken as a constant, the associated best $R_1$ is found. With $k_{2'\text{avg}}$ and $k_{2\text{app}}$ constant, and $C_R$ and $C_T$ known, the only unknown is $R_1$, which is found by simply rearranging the above equation to isolate it, as opposed to the mathematically similar but more difficult to immediately understand matrix manipulations required for multilinear matrix calculations.

The two step modifications of the simplified and full reference tissue algorithms significantly improve the noise-robustness of their algorithms for parametric imaging. Parametric images that appear essentially useless due to overwhelming numbers of bad voxels with a single-step three-parameter parametric imaging algorithm appear significantly improved when using a two-step process. It is also worth noting that, while this method can be applied to the simplified and full reference tissue models, it cannot be applied to the graphical Logan methods or EMIR because these methods estimate only the BP, and not the $k_2$, $R_1$ and/or $k_2'$, leaving nothing to fix between the first and second steps.

This method is a modification of the method developed by Wu and Carson (Wu and Carson, 2002). Their method also used a two-step process to calculate the $k_{2'\text{avg}}$ first, and then use that $k_{2'\text{avg}}$ to reduce the complexity of the individual voxel fits. Their method calculated the $k_{2'\text{avg}}$ using an average of the $k_2'$ results from individual voxels’ fits with the three parameter model, however, rather than first averaging the voxel TACs with an ROI as discussed above.
4.5 - Algorithm Comparisons

4.5.1 - Overview

The algorithms and models discussed above make different assumptions and use various implementation tricks to speed their processing and quality of results. Knowing these details does not immediately reveal which is best to use in any given imaging situation, however. Some predictions may be made, based on the assumptions and the nature of particular tracers, or expected amounts of noise, but to fully evaluate the methods, direct comparisons with real PET data are necessary. A significant portion of this work was to implement and test these methods.

The PET data used for these tests consists of images taken with the Siemens/Concorde Focus 120 microPET system. Images were taken of unilaterally striatally lesioned rats, with the tracers $^{11}$C-dihydrotetrabenazine (DTBZ), $^{11}$C-raclopride (Rac) and $^{11}$C-methylphenidate (MP). Images of twelve rats were used in this analysis, with approximately eight PET images per rat: four Rac scans, and two each of DTBZ and MP, although in some cases additional images were generated and used, and occasionally an image was omitted from analysis due to isolated problems with that image. Images were reconstructed on a 128x128x95 voxel grid, with voxels of size 0.43 mm by 0.43 mm in the transverse plane, and planes 0.8 mm apart.

For all PET images, ROI TAC binding potentials (BP) were calculated for the left and right striata with the cerebellum as a reference region. For algorithm comparisons, unlike the ROI placement method comparisons discussed earlier, ROIs were placed using mask guidance where the mask was registered to the PET images using the MNI register program. Mask placement in this manner was done as part of a separate study by another investigator, Katie Dinelle, though TACs were re-extracted and ROI averages of parametric images calculated as part of this work. For this analysis, 3 planes of striatal ROIs were used, which were 6 pixels wide and 8 pixels tall, or 48 square pixels and 8.0 mm$^2$. As well, 3 planes of cerebellar ROIs were used, which were 16 pixels wide and 6 pixels tall, or 96 square pixels and 16.0 mm$^2$.

BPs were calculated using the simplified Logan reference input graphical method, Gunn’s algorithm for the simplified reference tissue model, the linearized full reference tissue...
algorithm, the expectation maximization impulse response method and the modified graphical method.

<table>
<thead>
<tr>
<th>Algorithm</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simplified Logan</td>
<td>Most commonly used. Assumes target tissue compartments equilibrate after time $t^*$</td>
</tr>
<tr>
<td>Modified Graphical</td>
<td>Same assumptions as simplified Logan.</td>
</tr>
<tr>
<td>Gunn SRTM</td>
<td>Assumes target and reference tissues each act like single compartment.</td>
</tr>
<tr>
<td>Linearized FRTM</td>
<td>Assumes target tissue acts like two compartments, reference tissue like single compartment.</td>
</tr>
<tr>
<td>EMIR</td>
<td>Assumes target tissue compartments equilibrate after patch time, can be represented thereafter by single decaying exponential</td>
</tr>
</tbody>
</table>

Table 4.1 - Summary of reversible tracer kinetic modelling algorithms compared with ROI TAC inputs

Parametric images were generated using the same algorithms used on ROI TACs on individual voxel TACs for the simplified Logan, modified graphical and EMIR algorithms. Parametric images were also generated with the modified two-step Gunn simplified reference tissue algorithm and two-step linearized reference tissue algorithm. The two-step algorithms required ROI TAC input from the striatum, and the ROI TACs used were these same used for each scan for the ROI TAC BP calculations. Parametric images with all algorithms also required cerebellum ROI TACs for the reference tissue input function, which were the same ROIs and input function used in the ROI TAC BP calculations. After generating parametric images, the same striatal ROIs were used to average voxels' BP values, providing left and right parametric average BPs, to allow comparison with the ROI TAC calculations.
**Algorithm Notes**

<table>
<thead>
<tr>
<th>Algorithm</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simplified Logan</td>
<td>Identical equations as in ROI TAC case</td>
</tr>
<tr>
<td>Modified Graphical</td>
<td>Identical equations as in ROI TAC case</td>
</tr>
<tr>
<td>2-step Gunn SRTM</td>
<td>Two-step algorithm uses ROI TAC data to generate initial reference (k_2'), which is held fixed for second reduced-parameter parametric voxel fitting</td>
</tr>
<tr>
<td>2-step Linearized FRTM</td>
<td>Two-step algorithm uses ROI TAC data to generate initial reference (k_2'), which is held fixed for second reduced-parameter parametric voxel fitting</td>
</tr>
<tr>
<td>EMIR</td>
<td>Identical equations as in ROI TAC case</td>
</tr>
</tbody>
</table>

Table 4.2 - Summary of reversible tracer kinetic modelling algorithms compared for parametric imaging

### 4.5.2 - ROI TAC Algorithm Evaluation

ROI TAC BPs were calculated for unilaterally striatally lesioned rats using PET with the tracers DTBZ, MP and Rac. Individual rats were scanned several times, with each tracer being imaged at least twice per rat. Each image had two striatal TACs: one for the left, or unlesioned striatum, and one for the right, or lesioned stratum. Each striatal TAC, with a common cerebellum reference TAC, was analyzed with five algorithms: the simplified Logan graphical method, the modified graphical algorithm developed as part of this work, the linearized full reference tissue method (FRTM), Gunn’s algorithm for the simplified reference tissue model (SRTM), and the expectation maximization impulse response method (EMIR). Algorithm comparisons were done separately for each tracer.

For each TAC, the mean BP, \(BP\text{mean}\), was calculated from the BP estimates for all five analysis methods. The five-method mean was then subtracted from each single-method BP estimate, leaving the difference, \(BP_{\text{diff}}\), between each method's BP and \(BP\text{mean}\).
Algorithmically, this may be described:

For each tracer:
   For each TAC:
      For each method:
         Estimate BP
      End For
      Average 5 methods' BP estimates = BP_{mean}
      For each method:
         Subtract BP - BP_{mean} = BP_{diff}
      End For
   End For
   For each method:
      Average all TACs' BP_{diff} = BP_{bias}
      For each TAC:
         Subtract BP - BP_{bias} = BP_{scatter}
      End For
   End For
End For

BP_{scatter} is an indication of the "scatter" of each method's BP estimates, or the variation around the method's mean difference from the five-method means BPs. Each method's BP_{scatter} for each TAC is assumed to be a single sample of a random distribution of errors that each analysis method produces when estimating BPs. If many independently-measured TACs with the same "true" BP were available, when analyzed they would produce a distribution of BP estimates, which would be centred around the average BP estimate for that method. For the measured data in this work, the calculated "scatter" is a sampling of that distribution, after removing the constant average BP estimate, which is assumed to correlate well with the five-method average BP.

It is assumed that all five algorithms being tested are all estimating the BP with some independent random distribution of error, and that generating a BP is sampling the random error. By averaging different algorithms' BP estimates, errors are sampled five times – once for each algorithm – and the error of the average should be less than the average of errors of individual
BP estimates. This allows the five-method average to approximate the truth for the purposes of comparing the algorithms.

To characterize the degree of scatter about the unknown truth of each algorithm, approximated by the five-method average, the standard deviation of the scatter values for each scan and tracer with each algorithm were calculated, giving a figure of merit for the algorithm. Notably, calculating the standard deviation of $BP_{\text{diff}}$ for each method would yield the same result, but the calculation is done here with $BP_{\text{scatter}}$ values for clarity.

Additionally, the single-method BP estimates were plotted against the five-method mean BP for each TAC, separately for each tracer. Least-squares lines of best fit were determined from these plots, giving a correlation coefficient, intercept and slope for each method for each tracer. Ideally, methods will have high correlation coefficient with the mean, and the two will be related with a slope near one and an intercept near zero, which would indicate no systematic bias in an individual algorithm.

### 4.5.3 - Parametric Algorithm Evaluation

Parametric algorithms were evaluated first for the practicality of using the algorithm to produce parametric images. In particular, it is necessary that any algorithm executes in a reasonable amount of time on available hardware. Because parametric images comprise parameter estimates calculated from many single-voxel TACs, they must also be robust to noise, of which single-voxels TACs have much more than ROI-averaged TACs. All algorithms discussed in this work were able to produce reasonable BP estimates from ROI TACs, which were generally consistent between methods. When converted to produce BP estimates from single voxel TAC, however, differences in the usability of the algorithms became apparent.

### 4.5.4 - Statistical Comparisons

Parametric images were generated from rat PET images, with the tracers DTBZ, MP and Rac for the following algorithms: the simplified Logan graphical method, the modified graphical method, the two-step linearized full reference tissue model (FRTM), and the two-step Wu and
Carson algorithm for the simplified reference tissue model (SRTM). EMIR parametric images were not compared statistically due to the long processing times required per image. ROIs were placed on the parametric images, and averages of the parametric image within the striatal ROIs (one average each) was saved for each image as the final "parametric" BP estimate for the image and method. The ROIs used in this step were the same ROIs used to extract the striatal TACs used in the first step of the FRTM and SRTM algorithm to calculate $k_2'$. All methods—ROI TAC-based and parametric—used the same ROIs to generate ROI TACs in the striatum and cerebellum, and to average the striatal parametric image values.

Parametric image average BP values from within ROIs were compared with the corresponding ROI TAC BP values. For the standard and modified graphical methods, the averaged parametric image BP was compared with ROI TAC BP calculated using the same algorithm. The SRTM and FRTM algorithms were slightly different in the parametric and ROI TAC cases, as the ROI TAC versions were single-step and directly calculated three parameters: the BP, $k_2'$ and $R_1$. The parametric versions of these algorithms calculated the $k_2'$ using the ROI TACs, and then calculated BP and $R_1$ for each voxel while taking the first-step's $k_2'$ as a fixed constant, and then averaged the voxels' BP values to get a final parametric BP estimate. The parametric and ROI-TAC BPs were then compared, by taking the ROI-TAC BP as the assumed truth, and determining the average percent difference between it and the parametric BP.

4.5.6 - Execution Time

In addition to running successfully, algorithms must also run quickly enough to produce images in a reasonable amount of time. What qualifies as reasonable for image processing is very dependent on the available computer hardware and the needs of the examiner, however algorithms can be reasonable grouped into those which run in a few seconds per image, those which run in under one minute per image, and those which take over one hour per image. All algorithms tested in this work were implemented as MatLAB scripts, and run-time optimization was not a priority. Reimplementation as optimized compiled programs would likely improve runtimes significantly, although the general classifications by order of magnitude of runtime as outlined here are unlikely to be affected by such changes. Also, it is notable that the entire image volume is not processed by the algorithms discussed in this work. To speed parametric
image generation, the algorithms were restricted to a volume of voxels on the order of 45x45x40 voxels (with slight variations between methods because there was no reason to ensure they were exactly the same), compared to the full image volume of 128x128x95 voxels. This volume covered the rat brain, including the striata and cerebellum. Additional voxels outside this volume were not necessary for the comparisons discussed below, so were omitted to speed calculations.

4.6 - Algorithm Comparison Results

4.6.1 – ROI TAC Algorithm Evaluation

The scatter of each of the analysis methods Logan, SRTM, FRTM and EMIR for each of the tracers DTBZ, MP and Rac is shown in table 4.3.

<table>
<thead>
<tr>
<th></th>
<th>Simple Logan</th>
<th>Modified Graphical</th>
<th>FRTM</th>
<th>SRTM</th>
<th>EMIR</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTBZ</td>
<td>0.07</td>
<td>0.09</td>
<td>0.06</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>MP</td>
<td>0.03</td>
<td>0.04</td>
<td>0.02</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>RAC</td>
<td>0.05</td>
<td>0.08</td>
<td>0.05</td>
<td>0.02</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Table 4.3 - Standard deviation of individual algorithms' discrepancy ($BP_{scatter}$) in binding potential estimates from average of five methods.

Logan and the linearized full reference tissue algorithms had the most scatter. EMIR had moderate amounts, and the Gunn simplified reference tissue algorithm had the least.

Plots of single-method BP estimates against the five method mean demonstrate that all five methods generate nearly identical results in the ROI TAC case with all three tracers.
Figure 4.7 A - Plot of single method rat ROI BP estimates against the five method means for DTBZ with least-squares fitted lines with slope and intercepts shown.

Figure 4.7 B - Plot of single method rat ROI BP estimates against the five method means for MP with least-squares fitted lines with slope and intercepts shown.
The slopes and intercepts of the fits in the above plots of single method BP against five-method average BP are summarized in Table 4.4.

<table>
<thead>
<tr>
<th></th>
<th>Simple Logan</th>
<th>Modified Graphical</th>
<th>FRTM</th>
<th>SRTM</th>
<th>EMIR</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTBZ fit slope</td>
<td>1.01</td>
<td>1.00</td>
<td>0.98</td>
<td>1.00</td>
<td>1.01</td>
</tr>
<tr>
<td>fit intercept</td>
<td>-0.04</td>
<td>-0.01</td>
<td>0.05</td>
<td>-0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>MP fit slope</td>
<td>1.03</td>
<td>0.99</td>
<td>0.99</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>fit intercept</td>
<td>-0.02</td>
<td>0.01</td>
<td>0.03</td>
<td>-0.02</td>
<td>-0.01</td>
</tr>
<tr>
<td>RAC fit slope</td>
<td>1.04</td>
<td>0.98</td>
<td>0.96</td>
<td>1.01</td>
<td>1.02</td>
</tr>
<tr>
<td>fit intercept</td>
<td>-0.08</td>
<td>0.05</td>
<td>0.11</td>
<td>-0.04</td>
<td>-0.03</td>
</tr>
</tbody>
</table>

Table 4.4 - Slope and intercept of least-squares fitted line to plot of individual method BP estimates against five-method mean BP.

All methods’ intercepts are near zero, and all slopes are near 1, indicating that all methods agree well with the five-method average.
4.6.2 — Parametric Bad Voxels

When processing single-voxel TACs, the standard single-step Gunn solution for the simplified reference tissue model produced images in which many of the voxels had unreasonable BP estimates — often several orders of magnitudes larger than physiologically plausible — and inconsistent with most other voxels and the ROI averaged TAC results. These "bad voxels" — those in which the BP estimates are unreasonable - were sufficient in number to make the algorithms unusable in practice. In particular, bad voxels frequently appeared within the striatum, within the ROIs used to average parametric BP estimates.

![Figure 4.8 - Single-step parametric SRTM BP image of DTBZ rat brain, showing abundance of "bad voxels" in bright red](image)

Single-step linearized FRTM also produced bad voxels in parametric images. The number of bad voxels with single-step FRTM was significantly less than single-step SRTM, however. FRTM bad voxels were closer in value than SRTM bad voxels to the surrounding physiologically-reasonable voxel BP values, but were still inconsistent. FRTM bad voxels also occurred within ROIs placed on structures of interest, preventing ROI averaging of voxel BP estimates from producing useful average results, as occurred with SRTM.
Both FRTM and SRTM algorithms may be improved for parametric image generation by using the two-step variation. Having observed the necessity due to bad voxels in parametric images, these variations were investigated, and significantly fewer bad voxels were observed with both algorithms. Two-step parametric SRTM images again have fewer bad voxels than FRTM, although both have few enough – and none within the striatal ROIs – to allow the parametric images to be analyzed further.

Additionally, parametric EMIR was examined, and was found to have some bad voxels. There is no known way to modify EMIR to use a fixed reference $k_0'$ or other modification to improve its performance, so its characteristics in this regard are fairly compared to the best available (two step) versions of SRTM and FRTM. The number of bad voxels in EMIR is comparable to single-step FRTM, and some appear in or near structures of interest such as the striatum.
4.6.3 - Execution Time

When processing ROI TACs, all algorithms tested took less than one minute to produce BP estimates. ROI-based algorithms produced output seemingly instantly when running on a single TAC, or within a few seconds when running as part of a large batch of such calculations. Parametric image generation took significantly longer, with the graphical Logan methods, two-step simplified reference tissue method, and two-step linearized full reference tissue methods taking tens of seconds to run. The parametric expectation maximization impulse response method was much slower, however, taking five or more hours to generate a single parametric image with 30 iterations. This was too long to be able to generate parametric images for dozens of images, and is long enough to be prohibitively slow for practical use for image analysis. Given this speed limitation, and the presence of significant number of bad voxels in parametric images, the EMIR method was not compared further with other parametric methods.

<table>
<thead>
<tr>
<th>Algorithm</th>
<th>Execution Time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROI-TAC Algorithms</td>
<td>less than 5 seconds</td>
</tr>
<tr>
<td>Parametric Logan, FRTM (2-step), SRTM (2-step)</td>
<td>less than 30 seconds</td>
</tr>
<tr>
<td>Parametric EMIR</td>
<td>greater than 5 hours</td>
</tr>
</tbody>
</table>

Table 4.5 - Summary of execution times for kinetic modelling algorithms.

4.6.4 - Statistical Comparisons

<table>
<thead>
<tr>
<th></th>
<th>Logan</th>
<th>M-Graphical</th>
<th>FRTM</th>
<th>SRTM</th>
</tr>
</thead>
<tbody>
<tr>
<td>avg.</td>
<td>st. dev.</td>
<td>avg.</td>
<td>st. dev.</td>
<td>avg.</td>
</tr>
<tr>
<td>DTBZ unlesioned</td>
<td>-5.3</td>
<td>3.1</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>DTBZ lesioned</td>
<td>-7.9</td>
<td>5.8</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>MP unlesioned</td>
<td>-9.5</td>
<td>6.1</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>MP lesioned</td>
<td>-13.9</td>
<td>11.1</td>
<td>-0.2</td>
<td>0.8</td>
</tr>
<tr>
<td>Rac unlesioned</td>
<td>-3.8</td>
<td>2.3</td>
<td>-0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>Rac lesioned</td>
<td>-4.6</td>
<td>2.9</td>
<td>0.0</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Table 4.6 - Average percent difference between parametric and ROI-TAC BP estimates, and standard deviation of percent differences, for four kinetic modelling algorithms.

The noise-induced bias of the standard Logan algorithm (Slifstein and Laruelle, 2000) is seen here, with the noisier single voxel TACs producing an average BP between 4% and 14% lower than the corresponding ROI TAC. The FRTM and SRTM two-step algorithms show significantly smaller average bias with respect to their ROI TAC versions, with the FRTM
positive bias ranging from 1% to 4%, and the SRTM positive bias ranging from essentially 0% to 4%. Most notably, the modified graphical method has absolutely no bias observed between ROI TAC and parametric average BP in the DTBZ and unlesioned MP cases. In the lesioned MP and both Rac groups, average negative biases of magnitude less than 0.2% are observed. These biases are actually due to a very small number of individual ROI TACs which exhibit bias, while the majority of ROIs in the groups with nonzero average bias agree exactly between parametric and ROI estimates of BP. The five individual ROIs for which the ROI TAC and parametric BP estimates differed were produced from only three PET images; two images each had both striatal TACs with this irregularity, and one image had one TAC that differed, and one TAC which was consistent. It is unclear why a small number of TAC exhibit disagreement between ROI TAC and parametric ROI average. The five inconsistent TACs were visually inspected, but showed no obvious differences from consistent TACs. That two striatal pairs of TACs had the irregularity suggests that the common cerebellar TAC might be relevant to the problem, but nothing unusual was observed in those reference TACs.

4.6.5 - Discussion

In addition to average bias, the standard deviation of the individual scan changes from ROI TAC to parametric image average indicates the degree of variation around the mean bias. The standard Logan graphical method has a relatively large amount of scatter, which is likely due to the presence of $C_{T}(T)$, a single frame's target tissue TAC value, in the denominator of both the $X$ and $Y$ values used in the Logan formula. The single-frame measured value $C_{T}(T)$ is much noisier than its integral, so is the dominant source of noise in the expression.

The FRTM relationship also has unintegrated $C_{T}(T)$, appearing in the left hand side, or $Y$, value. Unintegrated $C_{T}(T)$ does not appear in the right hand side, or $X$, values however, limiting its overall influence on noise. Additionally, the Logan method calculates slope from $X$ and $Y$ values for the last half hour, or last four data points. These $X$ and $Y$ data points include both integrals from the start of the scan until the time of each data point of $C_{R}(T)$ and $C_{T}(T)$, respectively, and also the immediate values of $C_{T}(T)$ in their denominators. This gives relatively few samples of single frame noise in the fit, allowing random variations due to noise to have a larger impact on results. Conversely, FRTM fits are done to the full hour of scan integrated and
unintegrated data. This is contrary to the standard assumption that these compartmental models apply only after some time $t^*$ after which the reversible compartments have equilibrated, but is found to agree well with other methods, so is thought to be acceptable. Using significantly fewer data points with FRM is not feasible, as many as three independent parameters are being fit simultaneously, and too few data points with noisy data produces very unreliable fits.

The SRTM relations also use the integrals and immediate values of the full hour of TAC data. This is theoretically more problematic for SRTM than even FRTM, as SRTM is based on the assumption that the specifically bound compartment is very quickly equilibrated with the non-displaceable compartment, to the degree that they may be treated as a single compartment mathematically. Nevertheless, this method agrees well with Logan for ROI TACs. This may be due to the weighting factors which are used in the SRTM calculation, which adjust the significance of individual frames based on the number of true counts recorded during the frame (for the whole image volume) and the duration of the frames. The particular weight value used, duration$^2$/trues, where "trues" is the number of coincident photon detections during a frame, tends to cause weights for the last 6 or 8 frames to be significantly higher than the first few frames. The final four frames are weighted 100 times higher than the first six is possible, with middle frames intermediate between these extremes. This almost replicates the Logan graphical method's limit of using only the last four frames, at which point the reversible compartments should be equilibrated as assumed, but does incorporate earlier data, in order to ensure the fitted relationship is not completely inconsistent with earlier data.

The modified graphical algorithm replaces the $C_T(T)$ in the denominator of the standard Logan algorithm with $C_R(T)$. In parametric imaging, the $C_R(T)$ is still an ROI averaged value, which is significantly less noisy than the single-voxel $C_T(T)$. This effectively makes the denominator $C_R(T)$ a scaling factor on the X and Y values which are fitted by this algorithm. As the same scaling factor, $1/C_R(T)$, is used for the X and Y values for each time T, there is little change to that timepoint's contribution to the overall fitted slope. Unlike standard Logan, the scaling factor does not change from voxel to voxel in the parametric image, or between the voxels and the ROI TAC, which allows the ROI average of the parametric image and the ROI TAC BPs to be nearly identical, or exactly identical in some cases.
4.7 – Bad Voxel Investigations

4.7.1 - Causes of Bad Voxels

The presence of bad voxels—those which have unreasonable BP values compared to surrounding voxels—in parametric images raises the question of their source or cause. A particular voxel may be bad in the parametric image generated with one algorithm, but the same voxel may have a reasonable and consistent BP estimate in parametric images generated with another algorithm. From this, it is apparent that different algorithms may be more or less prone to producing bad voxels from the same TACs. By investigating the single-voxel TACs associated in the raw PET image that correspond to the bad voxels in parametric images, trends may be observed that suggest the mathematical features of the parametric imaging algorithms or TACs that cause bad voxels to appear.

For one DTBZ, one Rac and one MP parametric image, single-voxel ROIs were placed on two-step SRTM parametric rat brain images on bad voxels in those images. ROIs were copied to the corresponding 17-frame PET images, and used to extract representative bad voxel TACs for each tracer with SRTM. Bad voxel TACs for each tracer were averaged into a single TAC, and plotted.

![DTBZ SRTM 2-step Parametric Bad Voxels Averaged TACs](image)

Figure 4.11A – Average of several TACs corresponding to "bad voxels" in DTBZ 2-step SRTM parametric images.
As can be seen, the TACs corresponding to bad voxels have a tendency to increase during the last few frames. This is inconsistent with the assumption of a single decaying exponential being the dominant factor in the target tissue TAC at this time. When the algorithm attempts to fit a single decaying exponential to a TAC that is increasing at the end, it is forced to
use an exponential with a very small decay constant, so that the later – and heavily weighted in SRTM – timepoints of the estimated TAC are not well below the measured TAC.

Similar representative bad voxel TACs were not analyzed for the two-step FRTM algorithm because there were so few bad voxels in those parametric images. Although the same input data was used for all algorithms, FRTM was less prone to producing unreasonable BP estimates. Additionally, the majority of FRTM parametric image voxels that were inconsistent with other adjacent voxels were in regions of the image outside the brain, or even outside the whole rat body. In these locations, the assumptions justifying the compartmental model on which these algorithms are based likely do not apply, and there is little use in characterizing those voxel TACs, as they would never be included in analysis of a parametric rat brain image.

EMIR bad voxels were analyzed in a manner identical to that of SRTM. Parametric EMIR produced bad voxels similar in number to those that appear in two-step SRTM parametric images. As with SRTM images, single-voxel TACs were placed on bad voxels in an EMIR parametric image, and the corresponding single-voxel TACs were extracted from the raw PET image and averaged.

Figure 4.12 - Average of several TACs corresponding to "bad voxels" in DTBZ EMIR parametric images.
Once again, bad voxel TACs on average have an increase in the last few frames.

As with SRTM, EMIR as currently implemented assumes that the impulse response function will be a single decaying exponential after some time $t^*$ after which the reversible compartments have equilibrated. For voxels in which the noise random sampling has caused the later frames to have increases in measured activity, this assumption is not met, and inconsistent BP estimates can result. In more detail, when the later frame values of the target tissue TAC are small, the majority of the area of the impulse response function is in the earlier times. This occurs because reference tissue TAC and the target TAC both have similar shapes — initial rise followed by decay towards zero — and no significant area is needed in later times of the IRF. If, however, a target TAC has relative high values in the later frames, either due to actual lingering activity in the target tissue, or noise happening to be high at that time, the extra area near the end of the measured tissue TAC requires the IRF to also have higher values at the later times to fit the measured data.

When the unpatched impulse response has a relatively small area after the patch time, the fitted decaying exponential has a relatively large decay constant, and its area projected to infinite time is small, as its contribution to the total area under the IRF, and thus the BP. When the unpatched impulse response has relatively large values near the end of the calculated duration, fitting a decaying exponential gives a very small decay constant. In effect, the exponential is being forced to look like a non-decaying constant. When this near-constant decaying exponential’s area is projected to infinite time, the result is very large number. This large number can completely overwhelm the area of the IRF within the calculated time, and leads to the observed bad voxels in parametric images. This problem does not occur with ROI TAC calculations using EMIR because ROI TACs are the average of many voxel TACs, and are much less noisy, and do not exhibit the high or increasing values near their ends.

**4.7.2 - Bad Voxel Workarounds**

When the problem of bad voxels in parametric image generating algorithms became apparent, two methods were considered to work around the problem they presented, besides changing the parametric image generating algorithm. These methods were to selectively filter
out bad voxels from the ROI average of the parametric BP images, and to pre-blur the input image to reduce the amount of noise in the single (or now, several) voxel TACs, in the hope of reducing the appearance of noise-dependent effects that cause bad voxels. These methods were tried prior to developing the two-step parametric modelling techniques, and were tested on the single-step SRTM algorithm, which produced many bad voxels for with the same input images from which other algorithms produced few or no bad voxels.

Selectively ignoring bad voxels – defined as those with BPs above an arbitrary threshold – did allow ROI averaged BPs for parametric images to be calculated. Unfortunately, this method also biased the resulting BP average significantly. When ROI TACs are used as input to kinetic modelling, the noise of individual voxel TACs is averaged before modelling, so that the few voxels in which noise causes increases in the last few frames are balanced on average with the few voxels with unusually low values at later times. The averaged TAC has little noise-induced variation. When processing single-voxel TACs one at a time, however, only the voxels with high values of noise at the end of the TAC are cause bad voxels, and so only those TACs' BP values are removed from the average of voxels in a parametric image. The result is that there is a significant decrease in the calculated BP. This was observed when this method was tried with SRTM parametric images, and was then abandoned.

Filtering images prior to kinetic modelling for parametric image generation was also tested. This method has the advantage that it does not discriminate between high and low valued TACs, allowing averaging after parametric image generation to be unbiased. It does have a disadvantage however, in that the chosen filter – a blur – effectively reduces the image resolution. In fact, testing this method showed there to be a tradeoff between degree of blur, and thus resolution loss, and the effectiveness of the method at eliminating bad voxels. With more blur, the magnitude of bad voxels was reduced, making them more similar to surround voxels in BP value, but reducing the ability to discern smaller structure in the parametric image. Overall, this technique was judged inferior to using two-step SRTM for eliminating bad voxels.
4.8 - Rat Population Reference $k_2'$ Rate Constant

4.8.1 – Overview

With human data, literature values for population-average reference $k_2'$ rate constants are available to be used in the linearizing term of the standard Logan method. There are presently no available literature values of this rate constant for rats, however, so an alternative source was needed to be able to perform standard Logan analysis on large numbers of rat TACs in a manner consistent with the human Logan analysis, which uses a population reference $k_2'$. To generate a population $k_2'$ for rats, two methods were investigated: the SRTM and FRTM algorithms, both of which can be used to calculate $k_2'$ given target and reference TACs.

The linearized full reference tissue model was modified to output the reference $k_2'$ for each rat brain striatal TAC given as input. The method was run on the same set of rat TACs discussed above that were used to test the noise characteristics of the modified graphical algorithm. Two $k_2'$ values for each PET image, one for each of the lesioned and unlesioned striatal ROIs, were generated, and the average and standard deviation for each tracer was calculated. The averages gives a population-based estimate of the reference $k_2'$ for rats with each tracer, and the standard deviation gives an indication of how much variation there is within the tracer.

4.8.2 – Results

The results of calculating rat population reference (cerebellum) $k_2'$ values with FRTM are summarized in table 4.7.

<table>
<thead>
<tr>
<th>Tracer</th>
<th>$k_2'$ (unlesioned)</th>
<th>$k_2'$ st.dev. (unlesioned)</th>
<th>$k_2'$ (lesioned)</th>
<th>$k_2'$ st. dev. (lesioned)</th>
<th>$k_2'$ (all TACs)</th>
<th>$k_2'$ st. dev. (all TACs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rac</td>
<td>3.1</td>
<td>23.3</td>
<td>-0.01</td>
<td>0.71</td>
<td>1.6</td>
<td>16.5</td>
</tr>
<tr>
<td>DTBZ</td>
<td>-1.3</td>
<td>3.0</td>
<td>-0.2</td>
<td>1.4</td>
<td>-0.7</td>
<td>2.4</td>
</tr>
<tr>
<td>MP</td>
<td>0.25</td>
<td>2.21</td>
<td>-0.11</td>
<td>0.37</td>
<td>0.07</td>
<td>1.58</td>
</tr>
</tbody>
</table>

Table 4.7 - Average and standard deviation of estimates of reference cerebellum $k_2'$ for tracers Rac, DTBZ and MP in rat brains calculated using linearized FRTM algorithm. Values are in units of min$^{-1}$.

These $k_2'$ values were also histogrammed and plotted in figure 4.13.
Figure 4.13A - Histogram of FRTM $k_2'$ estimates with DTBZ. Number of estimates in each bin is plotted against bin number. 25 bins range from $k_2' = 0$ to $k_2' = 0.1 \text{ s}^{-1}$ (6 min$^{-1}$). Estimates created with unlesioned (blue) and lesioned (red) striatal ROIs are plotted separately.

Figure 4.13B - Histogram of FRTM $k_2'$ estimates with MP. Number of estimates in each bin is plotted against bin number. 25 bins range from $k_2' = 0$ to $k_2' = 0.1 \text{ s}^{-1}$ (6 min$^{-1}$). Estimates created with unlesioned (blue) and lesioned (red) striatal ROIs are plotted separately.
Figure 4.13C - Histogram of FRTM $k_2'$ estimates with Rac. Number of estimates in each bin is plotted against bin number. 25 bins range from $k_2' = 0$ to $k_2' = 0.1 \text{ s}^{-1} (6 \text{ min}^{-1})$. Estimates created with unlesioned (blue) and lesioned (red) striatal ROIs are plotted separately.

These reference $k_2'$ values are unsatisfactory. The standard deviation is significantly larger than the values themselves, and some of the values are negative, which is physiologically impossible. This indicates that the linearized FRTM algorithm is unsuitable for calculating population reference $k_2'$ values.

Rat population reference $k_2'$ were calculated with the SRTM algorithm. For DTBZ, the average reference $k_2'$ was $0.59 \text{ min}^{-1}$ with a standard deviation of $0.15 \text{ min}^{-1}$. For MP, the average reference $k_2'$ was $0.27 \text{ min}^{-1}$ with a standard deviation of $0.10 \text{ min}^{-1}$. For RAC, the average reference $k_2'$ was $0.38 \text{ min}^{-1}$ with a standard deviation of $0.09 \text{ min}^{-1}$.
Repeating this analysis with the SRTM algorithm produces better results.

<table>
<thead>
<tr>
<th>Tracer</th>
<th>$k_2'$ (unlesioned)</th>
<th>$k_2'$ st. dev. (unlesioned)</th>
<th>$k_2'$ (lesioned)</th>
<th>$k_2'$ st. dev. (lesioned)</th>
<th>$k_2'$ (all TACs)</th>
<th>$k_2'$ st. dev. (all TACs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rac</td>
<td>0.39</td>
<td>0.10</td>
<td>0.37</td>
<td>0.09</td>
<td>0.38</td>
<td>0.09</td>
</tr>
<tr>
<td>DTBZ</td>
<td>0.54</td>
<td>0.12</td>
<td>0.64</td>
<td>0.15</td>
<td>0.59</td>
<td>0.15</td>
</tr>
<tr>
<td>MP</td>
<td>0.24</td>
<td>0.06</td>
<td>0.30</td>
<td>0.13</td>
<td>0.27</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Table 4.8 - Average and standard deviation of estimates of reference cerebellum $k_2'$ for tracers Rac, DTBZ and MP in rat brains calculated using SRTM algorithm. Values are in units of min$^{-1}$.

These data were histogrammed, as seen in figure 4.14.

Figure 4.14A - Histogram of SRTM $k_2'$ estimates with DTBZ.
Figure 4.14B - Histogram of SRTM $k_2'$ estimates with MP.

Figure 4.14C - Histogram of SRTM $k_2'$ estimates with Rac. Number of estimates in each bin is plotted against bin number. 18 bins range from $k_2' = 0$ to $k_2' = 0.015$ s$^{-1}$ (0.9 min$^{-1}$). Estimates created with unlesioned (blue) and lesioned (red) striatal ROIs are plotted separately.
These results are significantly better than those generated with linearized FRTM. The standard deviations are at most 40% of the $k_2'$ values, the average values are positive and of reasonable order of magnitude, and the histograms more clearly show the values grouped around their average, unlike the well-spread FRTM histogram. As well, the lesioned and unlesioned ROI TAC estimates of reference $k_2'$ — which should be unaffected by lesioning — are within 20% of each other's values. These results allow rat population reference $k_2'$ values to be determined, so as to be used for rat implementation of the standard Logan algorithm. Comparison of standard and simplified Logan algorithms, as discussed below, demonstrate that these $k_2'$ are also large enough to justify using the simplified Logan with rats, rather than the standard Logan that is used with humans, who have a lower $k_2'$ than that of rats.

4.9 — Graphical Methods Comparisons

4.9.1 — Added Noise Overview

Additional testing of the modified graphical algorithm was done, to compare its performance to the standard Logan method with varying amounts of TAC noise. A rat brain DTBZ ROI TAC for the unlesioned striatum and the cerebellum were analyzed using the single-step SRTM algorithm, to produce an output BP, $k_2$ and $R_1$ as discussed above. The reference tissue TAC was then convolved with a decaying exponential and added, with suitable weightings, to the reference TAC, as indicated by the form of the SRTM algorithm, to calculate a simulated target tissue TAC.

\[ C_T = R_1C_R + k_2(1 - R_1/(1+BP))C_R \otimes e^{-k_{2app}t} \]

This calculated target TAC is used as a "noiseless" TAC with which to examine the effects of added noise on the two Logan methods. The reference tissue TAC is used unaltered, and the Gunn algorithm-derived $k_2$, $R_1$ and BP are discarded after this step.

The calculated target TAC is repeatedly analyzed with the two graphical methods (standard without linearizing term with reference $k_2'$, and the modified graphical method).
Standard Logan: \[ \int_0^T C_T(t) dt / C_T(T) = DVR \int_0^T C_R(t) dt / C_T(T) + \text{int} \]

Modified Graphical: \[ \int_0^T C_T(t) dt / C_R(T) = DVR \int_0^T C_R(t) dt / C_R(T) + \text{int}' \]

As discussed previously, and shown later in this work, rats have a relatively large \( k_2' \) and \( k_2 \), making it possible to omit these extra terms from the \( X \) values without significantly affecting the results with real rat data.

An initial iteration in which no noise is added to the target TAC is used to calculate baseline BP estimates for the two algorithms. Then, for a large number \( (10^6) \) of test cases, a Gaussian noise function is sampled and added to each frame of the target TAC. The two Logan algorithms are re-run with the noisy target TAC, and the resulting BPs are found for each and recorded. After all test cases are complete, the noisy BP values for each method are averaged and their standard deviation is calculated. Averages are expressed as a percentage of the original, noiseless BP. This entire process is repeated for several different Gaussian noise functions, with increasing variance in each set of tests. The final result of these tests is a series of BP biases and standard deviations as a function of noise function width (variance).

### 4.9.2 – Added Noise Results

Table 4.9 summarizes the results of adding noise on Logan methods' BP estimates.

<table>
<thead>
<tr>
<th>sigma (% max)</th>
<th>Simplified Logan</th>
<th>Modified Graphical</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BP Bias %</td>
<td>BP st.dev. %</td>
</tr>
<tr>
<td>0.4</td>
<td>-0.004</td>
<td>0.44</td>
</tr>
<tr>
<td>4</td>
<td>-0.38</td>
<td>4.4</td>
</tr>
<tr>
<td>8</td>
<td>-1.7</td>
<td>9.0</td>
</tr>
<tr>
<td>12</td>
<td>-4.3</td>
<td>13</td>
</tr>
<tr>
<td>16</td>
<td>-7.3</td>
<td>16</td>
</tr>
<tr>
<td>20</td>
<td>-9.8</td>
<td>18</td>
</tr>
<tr>
<td>40</td>
<td>-15</td>
<td>26</td>
</tr>
</tbody>
</table>

Table 4.9 - Bias and standard deviation of BP differences between noiseless and added-noise TACs analyzed with simplified Logan and modified graphical algorithms. Bias and standard deviation are expressed as a percentage of the noiseless BP. Variance of the added noise, sigma, is expressed as a fraction of the peak value of the noiseless TAC.
As can be seen, the modified graphical algorithm is significantly less prone to bias due to target TAC noise than the simplified Logan algorithm, but has similar overall variance in BP estimate due to noise. BP biases and standard deviations are expressed as a percentage of the “noiseless” initial TAC’s estimated BP. The added noise variance, sigma, is expressed as a percentage of the maximum frame value in the initial 17-frame input target tissue TAC.

Although not tested separately, it is likely that the modified graphical algorithm is more sensitive to reference TAC noise than is the standard Logan algorithm. This is because the modified graphical algorithm replaces the target TAC with the reference TAC in the expression in several places. In the case of rat striatum target TACs and cerebellum reference TACs, the reference ROIs are larger than the target ROIs, but the target region may have less statistical error due to larger activity within it. Switching to a method that is more sensitive to reference noise would thus be expected to increase the output BP scatter, as is seen when comparing the simplified Logan method to the modified graphical method results.

4.9.3 - Logan Methods Binding Potential Comparisons

While the noise characteristics of the modified graphical method are promising, they are insufficient to ensure the technique is useful for practical image analysis. It is also necessary for the method to produce reliable BP estimates. To test this, BP estimates generated with the modified graphical method, the standard Logan method without the linearizing term (as customary with rat data) and the standard Logan method with the linearizing term were all compared by plotting their BP estimates against the SRTM BP or the standard Logan BP. If there is good agreement with no bias between methods, then it is reasonable to use the modified graphical algorithm in cases where standard Logan would otherwise be used.

These tests were done using both human and rat data. Human PET data, with the same three tracers used on rats, was introduced to test the method's performance on this type of data. Humans have different brain neurochemistry than rats, and analysis methods may be more or less applicable to one, regardless of suitability to the other.
As before, the rat images were generated with a Siemens/Concorde Focus120 microPET system, with the tracer Rac, DTBZ and MP. Rat brain ROIs were placed using the atlas-guided methods discussed above. Rat Logan BPs were plotted against SRTM BP.

Human Parkinson's disease (PD) patients (Sossi et al., 2007) were scanned, as part of a separate study, with a Siemens/CTI ECAT 953B PET tomograph (Spinks et al., 1992). Patients were receiving chronic L-dopa treatment, and in some cases were taking direct DA agonists. All medications were stopped 12 to 18 hours before scans. Subjects were injected with 185 MBq of DTBZ, MP or Rac and scanned for 1 hour. Images were reconstructed with frames of durations 4 x 1 min, 3 x 2 min, 8 x 5 min and 1 x 10 min scans. A 10 min transmission scan using an external $^{68}$Ge source was performed before each emission scan and used for attenuation correction. ROIs were placed by experienced investigators using standard techniques for human images, the details of which are beyond the scope of this work. In human data, the target tissue is again the striatum, and the reference region is again the occipital cortex (for MP and DTBZ) or the cerebellum (for Rac), although in this work the occipital cortex TACs were used for all tracers. Using incorrect TACs in this manner is unlikely to invalidate results, however, as these regions TACs are expected to be similar, and the results are being compared between methods, not any external measurement or known truth. Human ROIs TACs were analyzed in the same manner as rat TACs, except that literature population values for reference $k_2'$ were available, and an initial step to determine $k_2'$ from SRTM was not necessary.

4.9.4 - Binding Potential Comparisons Results

The results of plotting multiple graphical methods' BP estimates are shown in figure 4.15.
Figure 4.15A - BP estimates for DTBZ rat TACs calculated with the standard (red) and simplified (blue) Logan algorithms and the modified graphical algorithm (green), plotted against SRTM BP.
Figure 4.15B - BP estimates for MP rat TACs calculated with the standard (red) and simplified (blue) Logan algorithms and the modified graphical algorithm (green), plotted against SRTM BP.

As can be seen, all three methods' BP estimates are scattered around the SRTM estimates, and are equivalent on average, with no method trending above or below the others. Amount of scatter is similar, with the modified graphical method possibly exhibiting less scatter in the MP plot.

Human graphical method BPs were plotted against the standard Logan BP, rather than the RPM BP, as the former is the established method to which the modified graphical is to be compared.
Figure 4.16A - BP estimates for DTBZ human TACs calculated with the simplified (blue) Logan algorithms and the modified graphical algorithm (green), plotted against standard Logan BP (red line).

Figure 4.16B - BP estimates for MP human TACs calculated with the simplified (blue) Logan algorithms and the modified graphical algorithm (green), plotted against standard Logan BP (red line).
Figure 4.16C - BP estimates for Rac human TACs calculated with the simplified (blue) Logan algorithms and the modified graphical algorithm (green), plotted against standard Logan BP (red line).

Notably, scatter apparent in these plots is not important. The plots could easily be remade with the modified graphical BP values on the horizontal axis, causing the modified graphical data points to appear as a straight line, and standard and simple Logan points to have scatter. Instead, in these plots, the significant observation is that the modified graphical and simplified Logan methods produce BP estimates which are significantly lower than those of the standard Logan method, particularly for larger BPs. This occurs because the standard Logan method’s linearizing term is equal to the reference tissue TAC at each frame, and is added to the integral of the reference tissue TAC. The relative significance of the added TAC value compared to its integral is larger for early time points, as the integral will always be increasing, but the single-frame TAC value will be large for early time points, and then decrease in later time points. Increasing the x components of data to which a line $y = mx + b$ is fitted, more so in the lower x values than the higher x values, has the effect of increasing the slope of the fitted line, leading to the standard Logan’s higher BP values as seen in figure 4.16.

More importantly, these human graphical plots demonstrate that the linearizing term present in the standard Logan method has a significant impact on the calculated BP values. Both
the simplified Logan and modified graphical methods omit this term, and exhibit similar
decreases in average estimated BP values. This is consistent with the human reference $k_2'$ being
small compared to that of rats. In rats, the large $k_2'$ and $K_1$ allow the reversible compartments to
equilibrating quickly, whereas with humans, more time is taken for equilibration to occur.
Equivalently, the linearizing term, which has $k_2'$ in its denominator, will be smaller for subjects
with larger $k_2'$. 
Chapter 5: Software Issues and Programming

5.1 – Data Processing

5.1.1 - Overview

A significant portion of this work consisted of dealing with various software issues and programming. Research-focused medical imaging software is of inconsistent quality, and often requires customisation to function as needed for an investigation. In particular, there are a wide variety of image formats used by medical imaging equipment manufacturers which have varying degrees and quality of support with existing analysis tools. In this work, it was necessary to write various scripts, programs, and program components to process data.

5.1.2 - ASIPro Image Loading and Saving

The ASIPro format is used by the Siemens/Concorde microPET Focus 120 system to store images. As ASIPro is a specialized image format, it is not natively supported by most image analysis software. During initial work with image registration and ROI placement, the MEDx image analysis and manipulation program was used to combine PET images with the rat brain atlas to produce masks, as well as do AIR registrations and reslicing of multi-frame images. To be able to use MEDx for these tasks, it was necessary to write ASIPro file format import and export scripts for MEDx. Parametric image generation was done primarily with MatLAB, but also c programs compiled to executables. In either case, ASIPro image loading and saving scripts were also required for these languages, and were written.

5.2 - Software Issues

5.2.1 - Discussion

Once loaded into image analysis software, ASIPro image analysis could commence. It was discovered that the analysis software had very significant bugs, however, which make the
programs useless for analysis, particularly for microPET images. The most drastic bugs were those with the MEDx and ASIPro programs' ROI averaging functions. In both programs, the user draws shapes or outlines on a displayed slice of images, and these shapes are intended to specify the location of ROIs used to average images. During this work, it was discovered that both MEDx and ASIPro were misrepresenting the location of ROIs with the on-screen shapes used to represent them. In both programs, the actual voxels averaged to generate an ROI value were frequently one or two voxels displaced from the displayed ROI position. This was verified by creating an ASIPro image which contained all zero voxels, except for a single nonzero voxel. ROIs were placed over and near this voxel, and ROI averages calculated. Frequently, an ROI would have a zero average despite the nonzero voxel being located within it, or a zero average when the voxel was outside the displayed ROI. The MEDx program remains in this bugged state due to developer inaction on reports of this bug, however correspondence with creator of the ASIPro program resulted in the majority of issues of this nature being resolved. This lead to the ASIPro program being used for the majority of TAC extraction in this work.

Another issue that arose was with the resampling algorithm used in the MEDx software to reslice images with transforms generated from AIR. To reslice a multi-frame PET image onto another image, the sum of all or a subset of the frames of these images were first coregistered using the in-GUI interface to AIR. While standard AIR produces a portable transformation file which can be easily applied to other images, MEDx hides this file from the user. Instead, MEDx has a facility where a transformation of a registered image may be saved and applied to another image. Unfortunately, the MEDx function that applies a transform does not have functional options to select the interpolation algorithm used to do the reslicing, and instead defaults to a trilinear interpolation algorithm. Trilinear interpolation can cause significant blurring of the image; in one test, reslicing an image and reslicing the result with the inverse transformation – so the final result was perfectly coregistered with the original image – and placing the same ROI on the rat striatum in both, the twice-resliced image had a 10% reduction in ROI average value. When determining quality of registration by differences in ROI average placed on the same structure, this introduces a significant source of error. It would also render any data analysis for experimental purposes less accurate, if one image was resliced and another not, due to the resliced image blurring. It was thus judged necessary to use another method to reslice images before taking ROI averages within them.
A promising alternative to MEDx for registration purposes is the MINC software package. MINC includes several separate programs which work together to provide a broad selection of image manipulation tools. The minctracc program, discussed above, is intended to perform image registration and includes options for algorithms including AIR and can apply masks to images when calculating registration transformations. In practice, however, minctracc is useless for most registration tasks because the program crashes or produces completely inaccurate registration transformations when non-trivial options are used. In particular, it was found that attempting to use masks – an essential feature for registrations in this work – was broken and unusable.

MINC does have a functional tool to reslice images given a transformation, however. The mincresample program has working options to set the interpolation algorithm. mincresample requires MINC-format transformation files as input, however, which cannot directly be produced with minctracc, as discussed above.

There is also a command-line version of the AIR program, which can be used instead of the MEDx GUI-accessed version. Command-line AIR is functional, in that all its options that are necessary for proper registrations to be done work correctly, however AIR is only able to take a few specific format images as input, all of which are signed integer formats. ASIPro images are 32 bit floats, and conversion to AIR-compatible formats proved difficult. Occasionally images would be output with reasonable registration, but the conversion between bit-depths was done incorrectly, and the output was unusable. As well, converting actual data images – not just the summed images used to calculate transformations – would have been necessary to be able to use the AIR-compatible reslicing programs, which is problematic in that it alters the actual data before analysis is completed, in a manner that directly limits the precision of the results.

One solution to this combination of partially-functional programs was to generate registered images with the AIR programs, in 16-bit signed format, using all the necessary registration options, and then use the simplest, and only functional, registration options in minctracc to register the original image to the AIR-resliced image. In this way, AIR determines
the transformation, and stored the information in the resliced image it produces. minctracc can then register the original image to its AIR-resliced counterpart without additional registration options such as masking, because the two images being registered cannot have the types of differences that motivated the use of masking when registering different images, as discussed above. After registering the original to the AIR-resliced image with minctracc, the transformation can be applied to the original image, which has not yet been altered, and produced useful output.

After this work was completed, another option became available, in the form of a version of AIR which outputs MINC-compatible transformation files directly. This eliminates the need to use an intermediate re-registration step, and is more reliable in practice.
Conclusions

Reliable ROI placement is essential for analysis of rat PET images of dopaminergic tracers. The cerebellum reference region and, in lesioned animals, the lesioned striatum do not appear distinctly in PET images. Employing a registered brain atlas to guide ROI placement as developed in this work makes these studies feasible. Atlas-to-PET image registration may be performed by manually specifying translations and rotations, or using point-picking software to generate a atlas-to-PET transformation. Atlas-guided ROI placement allows studies of heavily-lesioned rats that would otherwise not have been possible, and improves reference region ROI placement even in unlesioned animals.

When directly comparing PET images, automated coregistration methods such as AIR may be used to reslice images to match the spatial orientation of a target image. A common set of ROIs may then be used on all coregistered images. By employing a mask image that includes the activity concentrations in rat PET images that appear posterior to the eyes in the registration calculation, while excluding other areas outside the brain, the quality of automated registrations can be significantly improved. Using only the brain, without the activity concentrations posterior to the eyes, gives poor registration results. This method of coregistration, with ROIs placed on the registration target with atlas guidance, gives ROI placement that is as reliable as separately placing ROIs with mask guidance.

Equally as important as ROI placement is the method used to analyze time activity curves (TACs) extracted from PET images, either from ROIs, or for individual voxels in parametric imaging. More-accurate kinetic modelling methods allow smaller changes within and between images to be measured reliably, and eliminate biases that could distort results, which are important for PET imaging in which changes as small as 10% may be significant.

In this work, several kinetic modelling methods for dopaminergic tracers are tested on real rat PET data and compared. The methods include the Logan graphical method, the simplified reference tissue method (SRTM), the linearized full reference tissue method (FRTM), the expectation maximization impulse response method (EMIR), and a novel modified graphical method.
All methods produced essentially equal ROI TAC BP estimates on average, although the SRTM method had the least scatter, or variation around the consensus BP. Other algorithms have somewhat more scatter, with the modified graphical algorithm having the most for ROI TACs. The modified graphical algorithm was found to be not applicable to human data, because the $k_2$-dependent term is non-negligible in this case.

For parametric rat PET imaging, the two-step SRTM method has BP estimates that are relatively unbiased from the equivalent ROI TAC BP, and which have little scatter in differences between the parametric and ROI TAC BPs. Parametric two-step SRTM produces images with some “bad voxels” in which the estimated BP is unphysical and inconsistent with surrounding voxel BP estimates, however. Parametric EMIR also produces significant numbers of unphysical voxel BPs, and is extremely slow to run, making it impractical for analyzing many images with current computer hardware. The parametric two-step linearized FRTM algorithm generates few unphysical voxel BPs, although is more biased and has more scatter in differences between the parametric and ROI TAC BPs. The parametric Logan graphical method produces no bad voxels, however it is severely biased by the increased noise of single voxel TACs compared to ROI-averaged TACs, making it unsuitable for parametric imaging. The modified graphical method also produces no bad voxels, and has extremely low – often zero – bias between the ROI TAC and averaged parametric BP estimates, making the modified graphical method most suitable for use with rat parametric imaging.

Overall, the SRTM method was found to be optimal for analysis of ROI TACs of rat PET images for all dopaminergic tracers examined. For the parametric case, SRTM may also be used. However, the modified graphical algorithm developed in this work may be preferable, because it is the least sensitive to noise.
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