PET ASSAY OF EXTRASTRIATAL DOPAMINE D₂/D₃ RECEPTORS

by

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Abstract

Extrastriatal dopamine D2/D3 receptors have been implicated in a variety of cognitive and psychiatric disorders. \(^{18}\)F-Fallypride and \(^{11}\)C-FLB457 are commonly used PET radioligands for studying extrastriatal dopamine D2/D3 receptors, but differences in their \textit{in vivo} kinetics may affect sensitivity for measuring subtle changes in receptor binding. Focusing on regions of low receptor density, experiments were performed to compare the properties of \(^{18}\)F/\(^{11}\)C-fallypride and \(^{11}\)C-FLB457 in the rhesus monkey. Multiple-injection (MI) experiments were used to provide a full characterization of the \textit{in vivo} kinetic of both tracers, showing that \(^{11}\)C-FLB457 has a greater free space distribution volume than \(^{18}\)F-fallypride (\(V_{ND} = 3.0\) vs \(0.9\), respectively) and that \(^{11}\)C-FLB457 has a three-fold higher affinity for D2/D3 receptors (\(K_{D_{app}}=0.13\), FLB457; \(K_{D_{app}}=0.39\), fallypride). To investigate the sensitivity of both radioligands to changes in D2/D3 receptor density after drug intervention, we performed both receptor-blocking and dopamine depletion studies using \(^{11}\)C-fallypride and \(^{11}\)C-FLB457. D2/D3 receptor blocking studies with haloperidol show that both \(^{11}\)C-FLB457 and \(^{11}\)C-fallypride give similar measures of occupancy for the same drug dosage. Neither tracer was sensitive to changes after dopamine depletion with AMPT due to the bias introduced by using reference region methods. We also investigated the utility of using \(^{18}\)F-fallypride for measuring changes in D2/D3 binding due to deep brain stimulation (DBS) of the bed nucleus of the stria terminalis. These experiments measured large changes during scans acquired while the stimulators were on, and only small differences in scans where the stimulators were turned off, demonstrating that the use of high-affinity radioligands has great potential for advancing the understanding of the neurochemical changes induced by DBS. Taken as a whole, the experiments performed provide an evaluation of the differences between fallypride and FLB457, assessing their strengths and weaknesses for various imaging applications. This work provides a template for evaluating new tracers used for extrastriatal D2/D3 assay.
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Chapter 1

Introduction

Positron Emission Tomography (PET) imaging is a biochemically specific medical imaging modality that can non-invasively quantify the concentration of the brain’s dopamine D_2/D_3 receptors in vivo. PET methods are used to investigate dopamine's many roles, including modulation of motivation and reward, regulation of movement, and involvement with normal cognitive processing. Many neuropsychiatric disorders are believed to arise from abnormal dopamine levels and/or alterations in dopamine modulation. Dopamine receptors are mostly concentrated in the caudate and putamen, jointly referred to as the striatum, but they are also found at up to 100 times lower concentration in extrastriatal regions. Despite their relatively very low concentration, these extrastriatal dopamine receptors play a large role in normal functions such as working memory, cognition, and emotional processing but also in disease states such as schizophrenia, drug abuse, and attention deficit hyperactivity disorder.

1.1 Dopamine receptor distribution and physiology

Dopamine (DA) receptors can be categorized in two categories: D_1-like (excitatory; D_1, D_5) and D_2-like (inhibitory; D_2, D_3, D_4). Located exclusively postsynaptically, D_1-like receptors are found primarily in the hippocampus and cortex, while D_2-like (D_2/D_3) receptors are concentrated primarily in the caudate and putamen, both presynaptically and postsynaptically. Quantification of D_2 receptor densities has been measured in post mortem human brains, using^{125}I-epidepride, finding a range of concentrations over two orders of magnitude, from 16.6±3.6 pmol/g tissue in the putamen to 0.17±0.06 pmol/g in the middle frontal gyrus (Kessler et al 1993). The highest concentration of cortical D_2 receptors was found in the temporal cortex, with a concentration of 0.44±0.11 pmol/g. Striatal receptors receive dopaminergic
innervation from the substantia nigra via the nigrostriatal fiber tract and while extrastriatal areas receive projections from the ventral tegmental area via the mesolimbic tract (limbic system) and the mesocortical tract (neocortex). Receptors in the pituitary receive dopaminergic innervation from the arcuate nucleus of the hypothalamus via the tuberinfundibular system.

Research has shown that dopamine and extrastriatal D<sub>2</sub>/D<sub>3</sub> receptors play an important role in normal cognitive processing, with special emphasis on working memory. Early work by Goldman-Rakic et al. provided a link between working memory and DA (Goldman-Rakic 1990). Additionally, connections between DA and high level cognitive processes have been realized in both rodents and non-human primates (Arnsten 1998; Cropley et al 2006; Watanabe et al 1997). Fried et al. extended the work with animal models to humans subjects, finding an increase in DA levels in the amygdala during a cognitive task, as measured semi-invasively using microdialysis (Fried et al 2001). Using PET, others have indirectly measured significant changes of dopamine in extrastriatal areas during a task through measuring a change in competitive interactions between endogenous dopamine and radioligand (Aalto et al 2005; Christian et al 2006).

Beyond the role of dopamine and extrastriatal D<sub>2</sub>/D<sub>3</sub> receptors in normal cognitive processes, these receptors are also implicated in psychotic diseases such as schizophrenia. The dopamine hypothesis in schizophrenia originates from the discovery that chlorpromazine was effective in treating schizophrenia (Laborit and Huguenard 1951), which also led to the discovery of a variety of similar antipsychotic medications, all of which bound to D<sub>2</sub>-like receptors and not to D<sub>1</sub>-like receptors (Seeman et al 1975). The work of Weinberger et al. suggests a more specialized hypothesis of schizophrenia which stems from hyperactivity of the mesolimbic pathway and hypoactivity in the mesocortical pathway, both of which
have projections outside of the striatum (Weinberger 1996). As measured by SPECT using a dopamine 
depletion experiment, it has been reported that patients with schizophrenia have increased baseline D₂/D₃ 
occupancy (Abi-Dargham et al 2000).

Furthermore, dopamine and its D₂/D₃ receptors are intimately linked with movement disorders. A 
common movement disorder, Parkinson’s Disease (PD), is characterized by a degradation of 
dopaminergic neurons in the nigrostriatal pathway, but extrastriatal dopaminergic pathways are also 
degraded. Kaasinen et al. have used in vivo PET imaging to find that in some extrastriatal regions, D₂/D₃ 
density is decreased in advanced PD, and that the rate of loss of D₂/D₃ receptors is faster in extrastriatal 
regions than in the striatum (Kaasinen et al 2000; Kaasinen et al 2003). These results may help explain 
why cognitive and emotional disorders may be associated with PD (Tamaru 1997).

Considering dopamine’s implicated role in both normal and diseased states, development of methods for 
non-invasive measurement of dopamine function is important. With improved methods stemming from 
greater knowledge of radioligand characteristics, PET imaging studies can be better designed with greater 
sensitivity to biological changes, hopefully leading to a better understanding of dopamine function in 
both normal and disease states. With further research, this could also lead to more effective methods of 
diagnosis and treatment across a wide range of neurological disorders.

1.2 PET imaging of extrastriatal D₂/D₃ receptors

Since its onset, PET imaging has been used in neuroligand imaging, with many applications investigating 
dopamine receptors. The first example of this work was performed at Johns Hopkins University, where
Wagner et al. showed the feasibility of using PET to image dopamine receptors in vivo using $^{11}$C-NMSP, a methylated analog of $^3$H-spiroperone, a known antipsychotic drug which has long been used for in vitro pharmacology experiments (Wagner et al 1983). Following this initial study, research continued with $^{11}$C-NMSP, but other work was being done to investigate the utility of radio-labeled analogs of other antipsychotics, including remoxipride, which led to the discovery of raclopride (de Paulis 2003). Demonstrating its utility for in vivo imaging of dopamine receptors, initial in vivo studies with $^{11}$C-raclopride showed 4/1 ratio of striatum/cerebellum as well as specificity for D$_2$/D$_3$ receptors (Farde et al 1985).

While the discovery of raclopride has led to volumes of research on striatal D$_2$/D$_3$ receptors, its low specific-to-nondisplaceable binding ratios make it ill suited for imaging extrastriatal dopamine receptors (Farde et al 1988). As to improve on the low specific-to-nondisplaceable binding ratio of raclopride, other imaging agents were developed with a higher affinity for D$_2$/D$_3$ receptors, a characteristic needed for imaging of these receptors in extrastriatal regions of the brain (affinity = 1/K_Dupp). Amongst other candidate molecules derived as analogs to remoxipride, fallypride and FLB457 (Figure 1-1) emerged as high affinity D$_2$/D$_3$ receptor antagonists that could be labeled with positron emitting isotopes. Fallypride was first developed by Mukherjee et al. while at the University of Chicago, demonstrating desirable target to cerebellum ratios in extrastriatal regions, as well as the ability to be displaced by haloperidol (Mukherjee et al 1995; Mukherjee et al 1999). FLB457 was first synthesized in 1983 by Astra Pharmaceuticals, but many years passed until it was used as a PET radioligand for imaging extrastriatal D$_2$/D$_3$ receptors (Halldin et al 1995). Since their discovery, both radioligands have been used in many PET experiments with a variety of goals ranging from basic methods development to evaluation of neuropsychiatric disorders in patient populations.
Figure 1-1: Molecular structures

Both structures are classified as substituted benzamides

With PET, neuroreceptor imaging protocols often aim to detect a difference in receptor density across groups, between conditions, or as brought about by a pharmacological intervention or behavioral task. In a standard, single injection PET experiment, the metric of interest is often the nondisplaceable binding potential, which is an index of ligand-receptor interaction \( \text{BP}_{\text{ND}} = \frac{k_{\text{on}}B_{\text{max}}}{k_{\text{off}}} \). Considering endogenous dopamine is known to compete with radioligand for available binding sites, a change in nondisplaceable binding potential can imply a change in receptor density as brought about by changes in the concentration of synaptic dopamine, radioligand-receptor affinity, and/or receptor density.

Experiments designed to examine changes in binding potential within a single subject can be categorized into two types; (1) blocking studies and (2) challenge studies. A blocking experiment is performed in two separate scanning sessions, the first providing a measure of baseline \( \text{BP}_{\text{ND}} \), and the second gives a \( \text{BP}_{\text{ND}} \) after a change in condition. A challenge study, differs from a blocking study in that it is performed in a single scanning session with the origination of change in binding potential (i.e. amphetamine administration or task initiation) coming mid-way into the scanning session. After a manipulation
inducing an increase in synaptic dopamine, the PET signal will be diminished as the number of receptors available for radioligand binding is decreased.

Recently, there have been a number of papers examining the sensitivity of these high-affinity radioligands to detecting a change in extrastriatal endogenous dopamine levels using the blocking-type study design, each resulting in seemingly contradictory results. Using $^{11}$C-FLB457 in humans, Montgomery et al. reported a drop in cortical distribution volume ($V_T$) after methyphenidate as compared to baseline, but only when using arterial blood sampling methods (Montgomery et al. 2006). Using similar methods involving measurement of the arterial plasma input function; however, Aalto et al. were not able to detect a significant drop in BP$_{ND}$ after administration of d-amphetamine (Aalto et al. 2009). Using $^{18}$F-fallypride, Riccardi et al. report significant decreases in BP$_{ND}$ in the temporal cortex and other regions after a low dose of d-amphetamine (Riccardi et al. 2005). Most recently, Narendra et al. compared the sensitivities of $^{11}$C-FLB457 and $^{11}$C-fallypride to detecting amphetamine-induced dopamine release, concluding that $^{11}$C-FLB457 is more sensitive in this ‘blocking’ type experiment. One of the motivating factors to this work is to provide a comparison of the kinetics of fallypride and FLB457 as to be able to better predict which is more sensitive to changes in both ‘blocking’-type and ‘challenge’-type experiments.

Single injection experiments without arterial blood sampling often use reference tissue methods as a way to estimate nondisplaceable binding potential by comparing the degree of binding in receptor-rich tissue relative to a reference region. In this model, the reference region is assumed to be void of receptors. In dopamine receptor imaging, the cerebellum is commonly used as the reference region; however, there have been numerous reports that dopamine receptors are found at a non-negligible concentration in the cerebellum, violating an assumption inherent to the reference region model (Asselin et al. 2007; Pinborg et
This violation leads to an underestimation of nondisplaceable binding potential. In this work, we investigate the implications of this bias in nondisplaceable binding potential with both fallypride and FLB457.

While a standard single-injection study can provide a binding potential measure, there are techniques to determine more information about the radioligand-receptor interactions. For instance, through giving multiple injections of radiotracer at varied specific activities, multiple-injection (MI) protocol can determine all of the kinetic parameters of a two-compartment kinetic model (Figure 1-2) in a single scanning session. These parameters include those related to plasma transport ($K_1$ and $k_2$), binding and dissociation ($k_{on}$ and $k_{off}$) and receptor density ($B_{max}$). This increased knowledge about tracer kinetics from a single experiment is valuable, but comes at the cost of being a much more complicated experiment to design and implement, making it impractical to consider using for a large number of subjects. Furthermore, as it involves injection of low-specific activity radioligands leading to receptor saturation, this technique is best suited for use in animal models rather than in human subjects. This work uses the multiple injection technique to fully characterize the in vivo kinetics of $^{18}$F-fallypride and $^{11}$C-FLB457 in the rhesus monkey.

![Figure 1-2: Standard two-compartment model](image)
1.3 Experimental observations of a change in binding potential

Across the wide variety of methods used for PET imaging, including PET neuroreceptor imaging, the goal of many experiments is to detect changes in or a difference in the underlying physiology of two or more groups of subjects, as measured through a change in radioligand binding. The underlying cause of such a change can be due to a number of mechanisms, including drug interactions, disease states, behavioral changes, or electrophysiological manipulations. In this work we induce changes in radioligand binding in two ways. First, we investigated the differences in both $^{11}$C-FLB457 and $^{11}$C-fallypride in drug manipulation experiments. Second, we used $^{18}$F-fallypride for measurement of changes induced via electrical stimulation of neurons.

1.4 Thesis organization

The following chapters are organized with the goal of presenting the technical aspects of the work first, transitioning through a comparison of FLB457 and fallypride, then concluding with a study applying PET imaging techniques to another field of biomedical research, deep brain stimulation. Chapter 2 presents a description of the instrumentation that made the rest of this work possible and continues by describing the methods used in the radiosynthesis of $^{11}$C-FLB457, $^{11}$C-fallypride, and $^{18}$F-fallypride. Next, Chapter 3 describes the multiple-injection studies we performed with $^{11}$C-FLB457 and $^{18}$F-fallypride, concluding that the main differences of these ligands are in their $\text{in vivo}$ affinity and their tissue-to-plasma efflux constant, $k_2$. Through further experiments and computer simulations, Chapter 4 examines the differences in tracers when a drug manipulation is used to affect regional binding with a focus on how these methods are effected by non-negligible cerebellar binding. In Chapter 5, we demonstrate that quantitative PET neuroimaging can be very sensitive to the changes as brought about by deep brain stimulation. Chapter 6
gives a conclusion to this work emphasizing the importance of this research and suggesting what other types of studies could be done to build upon the work presented herein.
1.5 References


Laborit H, Huguenard P (1951) [Artificial hibernation by pharmacodynamical and physical means.]. Presse Med 59


Weinberger DR (1996) On the plausibility of "the neurodevelopmental hypothesis" of schizophrenia. *Neuropsychopharmacology* 14
Chapter 2

Radiochemical Production & Instrumentation

2.1 Introduction

This chapter gives details regarding the radiochemical synthesis of the three tracers used in this work, $^{11}$C-FLB457, $^{11}$C-fallypride, and $^{18}$F-fallypride. As needed for both carbon-11 tracers, the carbon-11 intermediates ($^{11}$C-methyl iodide and $^{11}$C-methyl triflate) are synthesized from $^{11}$C-$\text{CH}_4$, as produced in a gas target (90% N$_2$, 10% H$_2$) via the $^{14}$N(p,$\alpha$)$^{11}$C reaction on either the Waisman Center’s 6 MeV 9SDH-2 tandem linear accelerator (National Electrostatics, Middleton, WI) or the 11 MeV RDS 112 (CTI, Knoxville, Tennessee). For production of $^{18}$F-fallypride, $^{18}$F-flouride was produced via the $^{18}$O(p,n)$^{18}$F reaction with 11 MeV proton irradiation of a liquid target of 95% enriched H$_2^{18}$O on the RDS 112 cyclotron. While descriptions of methods of production of $^{11}$C-$\text{CH}_4$ and $^{18}$F-flouride at high specific activity have been provided previously (Barnhart 2004; Nye 2005), a description of changes in the control system of the RDS 112 cyclotron and a description of methods for conversion from $^{11}$C-$\text{CH}_4$ to $^{11}$C-$\text{CH}_3$I are provided herein.

2.2 A new control system for the RDS 112

2.2.1 Motivation for change

CTI’s prototype RDS-112 cyclotron was installed on campus at the Medical Science Center in 1985. Since its installation, its proven ability to produce a wide variety of radionuclides (Nickles 1991) has created a demand for reliable radioisotope production for researchers both on campus and across the
country. For its first 20 years, production demands for isotopes were met while operating the cyclotron using the original computer-interface hardware, consisting of an STD bus interfacing with a PC running the iRMX operating system. By 2004, there was growing concern that the computer control system may soon fail, making the cyclotron unusable. It was determined that the ways to ensure dependable cyclotron operation were to either take a passive approach, stocking up on compatible parts and repair the PC as needed; or a more proactive approach, replacing the aged computer before problems arise. In evaluating the advantages and disadvantages of these options, it was decided that a redundant system should be implemented: A new computer interface would be built. It would reproduce and improve upon the functionality of the original system while the original system would remain in-place, with switching between the new and old systems to be accomplished with minimal effort.

2.2.2 Description of original I/O system

In the original control system the STD bus (WinSystems, Arlington, TX) was the central hub of the cyclotron’s control system. Signals to and from various electronics components of the cyclotron are routed into the STD bus via six ribbon cables connected to terminal blocks TB2 through TB5 and through digital input output boards DIO1 and DIO2. The STD Bus also has connections to the iRMX PC and the local control chassis (LCC) via two 40-conductor ribbon cables. Each of the ribbon cables passing into the STD Bus was connected to a separate data I/O board, providing an interface between the LCC/PC and the cyclotron.

A diagram of the connections in the system with the STD Bus is shown below in Figure 2-1 with descriptions of the signals carried over each cable given in Table 2-1.
2.2.3 Description of new I/O system

The role of the PC and STD bus in the original system was replaced by a custom-built PC based running Windows XP. The PC resides in a rack mount server case, installed in the cyclotron’s control cabinet, immediately below the STD bus. Functionality of the hardware of the STD bus was replaced by hardware built by National Instruments (NI), which interfaces with LabVIEW software (Austin, TX). The components of the computer system include 1 GB of RAM, dual 80 GB hard drives configured with
RAID 1 (mirroring) to prevent downtime due to hard drive failure, a AMD Sempron 3100+ Processor, and a DVD writer for data backup purposes. The Bluetooth wireless keyboard and mouse have up to a 25’ range so they can be used anywhere in the control room.

To aide in switching between new and old control systems, all signals were still routed through the original set of terminal blocks and digital I/O boards along with the associated cables. However, since the hardware in the STD bus and the NI hardware have different connector types and pinouts, cables from TB2-TB5 and from DIO1, DIO2 that originally went to the STD bus are now routed to a custom connector block interface, routing signals from the terminal blocks to the appropriate channel on the new data acquisition (DAQ) hardware. Figure 2-2 diagrams the interconnections of the new system, and Table 2-2 gives descriptions of the signals routed to the DAQ hardware.
Figure 2-2: Connection diagram for new system with new PC

Table 2-2: New system cable descriptions

<table>
<thead>
<tr>
<th>DAQ card</th>
<th>Card Description</th>
<th>Signals Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCI-6033 (A)</td>
<td>100 kS/s, 16-Bit, 64-Analog-Input Multifunction DAQ</td>
<td>Counter: APS frequency</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Differential analog inputs (i.e. APS CR)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Referenced analog inputs (i.e. grid bias VR)</td>
</tr>
<tr>
<td>PCI-6033 (B)</td>
<td>100 kS/s, 16-Bit, 64-Analog-Input Multifunction DAQ</td>
<td>Analog measurement of current from quad slits</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TC1 and TC2 signals, differential analog</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Counters: Neutrons and Gammas</td>
</tr>
<tr>
<td>PCI-6059</td>
<td>96-channel 5 V TTL/CMOS Digital I/O</td>
<td>Digital inputs (i.e. power readouts)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Digital outputs (i.e. target valves, power control)</td>
</tr>
<tr>
<td>PCI-6703</td>
<td>Static Analog Voltage Output</td>
<td>Analog output (gas flow control)</td>
</tr>
</tbody>
</table>
The software, as written in LabVIEW, provides the interface between the user and the cyclotron. Each input/output channel is effectively polled at a rate of 1Hz. Analog channels are sampled numerous times within each sampling period as to average out signal noise. Each voltage measurement is scaled according to original design specifications, recorded, and logged to creating a history of the signal over the time course of a production shot. Detector count rates are determined through counter hardware. In software, the counters are initialized to 0 when the program starts and count rate is calculated by dividing the difference in counter values between time points by the time between samples (nominally 1 second). Every second, the value for each channel is displayed on the front panel (read channels) or output to hardware (write channels). Shutting down the program gives the option to save the history of all data values. The user interface (front panel) of the program, is shown below in Figure 2-3.
2.2.4 Added functionality

During cyclotron production of radioisotopes, it is of great interest to gather as much information about beam-target interactions as possible as well as to monitor the state of the different components of the cyclotron. Supplementing the information recorded in laboratory notebooks, the new software creates a log of all measured values, and plots them in a Microsoft Word file, creating a comprehensive log of conditions of the cyclotron for the duration of all runs.
Another function that has been integrated into the new control program is the inclusion of neutron and gamma counter data, which can be valuable indicators of the rate of isotope production, especially in the case of the $^{18}\text{O}(p,n)^{18}\text{F}$ reaction (Nickles et al. 1985). With this new system, the counter values are logged with their history displayed on the front panel of the control software, rather than having only instantaneous measures available. Displaying a graphical history of count-rates allows the operator to more easily identify trends as opposed to reading recorded values from a lab notebook.

In addition to simply logging counts from radiation detectors and analyzing trends, a measure that is of particular interest is that of the leaky-integrator of neutron flux (Nickles et al. 1985; Snowdon 1950; Votaw et al. 1987). A leaky integrator in this application provides an estimate of in-target radioactivity. While leaky integrators have been previously implemented for the purpose of tracking radioactive buildup using digital hardware (Nickles and Hichwa 1979), implementation in software is much simpler: The value of the leaky integrator at any time point is the sum of the counts collected in the previous sampling interval and the previous value decay corrected for the duration of the sampling period, as given below.

$$\text{Int}_{\text{Leaky}}(t) = \text{counts} + \text{Int}_{\text{Leaky}}(t-\Delta t)e^{-\lambda\Delta t}$$

This value is logged and displayed on the same chart as the gamma and neutron count-rate history. Monitoring this plot can be of particular interest in long shots producing non-standard radionuclides, during $^{18}\text{F}-\text{F}_2$ irradiation, and when the cyclotron provides inconsistent beam current.
2.2.5 Impact of new control system

The conversion from the new to old system was relatively simple. As the new system was being developed, cables were switched back and forth between systems with ease for testing of the new system, then back to the original for production runs. After proper validation, the new system was run as the primary system, starting in the summer of 2005.

This system has met its goal of providing a reliable control system for the RDS 112 (the only instances of computer-related down-time were related to processor overheating due to a failed cooling fan). Furthermore, the reliable nature of the new control system allowed for radioisotope production for research described in the body of this work. Considering its functionality over the past few years, the progression of computer hardware, and National Instrument’s commitment to support their hardware, it is likely that this new control system could continue to function for the remainder of the lifetime of the RDS 112.

2.3 Production of $^{11}$C-CH$_3$I and $^{11}$C-MeOTf

**Background**

Many $^{11}$C labeling methods have relied on the use of $^{11}$C-methyliodide ($^{11}$C-CH$_3$I) as a reactive intermediate in labeling precursor molecules. $^{11}$C-CH$_3$I was first synthesized using the ‘wet method’ which involved conversion of $^{11}$C-CO$_2$ to $^{11}$C-CH$_3$OH using LiAlH$_4$ (Crouzel *et al* 1987; Marazano *et al* 1977). The $^{11}$C- CH$_3$OH is then converted to $^{11}$C-CH$_3$I in solution via a reaction with aqueous HI. Unless great care is taken to prevent the introduction of atmospheric CO$_2$ into the system, the specific activity of the $^{11}$C-CH$_3$I will be reduced by $^{12}$C-CO$_2$ absorption onto the LiAlH$_4$. Furthermore, this reaction requires
that all reaction vessels need to be carefully cleaned before each run, which can lead to considerable radiation exposure to the operator during back-to-back runs.

In 1995 Link et al. (Link et al 1995) and Larson et al. (Larsen et al 1995) both proposed an easier, more robust route to methyl iodide production through gas phase chemistry, eliminating the need to clean the system between runs and being less vulnerable to atmospheric CO₂ (Larsen et al 1995; Link et al 1995). Both methods rely on the radical reaction of I₂ vapors with ¹¹C-CH₄ gas at ~700°C, producing ¹¹C-CH₃I (Larsen et al 1997; Link et al 1997). The ¹¹C-CH₃I is then trapped until released, and in the case of Larsen’s method, the ¹¹C-CH₄ makes multiple passes through the reaction oven in a recirculating loop, increasing yields. These gas phase methods have yields reported up to 80%, and at higher specific activities than with the ‘wet’ method.

With ¹¹C labeling chemistry, a common labeling method is to bubble ¹¹C-CH₃I gas into a solution containing precursor, then wait for the solution to react, followed by separation via high performance liquid chromatography (HPLC). Researchers have sought improve the process by a variety of methods. An example of such a modification is the on-loop labeling method, where the precursor is deposited on the walls of an HPLC injection loop rather than in solution (Studenov et al 2004). Another modification involves the conversion of ¹¹C-CH₃I to ¹¹C-MeOTf (¹¹C-methyl-triflate, ¹¹C-CH₃OSO₂CF₃). As ¹¹C-MeOTf is more reactive intermediate molecule than ¹¹C-CH₃I, this leads to shorter reaction times, lower reaction temperatures, and higher radiochemical yields (Jewett 1992).

The production of ¹¹C-CH₃I on campus has been evolving since spring 2003, where the first attempt at the
gas phase conversion of $^{11}\text{C-CH}_4$ to $^{11}\text{C-CH}_3\text{I}$ was done at UW-Waisman Center (Vandehey et al 2004). Since then, the rig has evolved from a manually controlled single-pass system to a LabVIEW-controlled recirculating system, now in its 3rd generation. As diagrammed in Figure 2-4, the current rig is in its most reliable and most simplified state yet, consisting of only five valves, a single porapak trap, and an add-on module built for converting $^{11}\text{C-CH}_3\text{I}$ to $^{11}\text{C-MeOTf}$. Production yields range from 50-75% (decay corrected, $^{11}\text{C-CH}_4$ to $^{11}\text{C-CH}_3\text{I}$) with a conversion time of 5-8 minutes.

**Figure 2-4:** Schematic Diagram of $^{11}\text{C-CH}_3\text{I}$ rig.

The $^{11}\text{C-CH}_4$ trap is inserted into a closed loop, then the pump drives recirculation of gases through reaction oven and $^{11}\text{C-CH}_3\text{I}$ is trapped. After recirculation, $^{11}\text{C-CH}_3\text{I}$ trap is heated, releasing $^{11}\text{C-CH}_3\text{I}$ through the triflate furnace (optional) en route to bubbling into a precursor solution.
The $^{11}\text{C}$ radiochemistry described in subsequent sections has been performed with $^{11}\text{C}-\text{CH}_3\text{I}/^{11}\text{C}\text{-MeOTf}$ produced originally on the automated rig described above with $^{11}\text{C}\text{-CH}_4$ being delivered from RDS 112 across campus. Since early 2008, however, a commercially available radiochemistry rig has been used for $^{11}\text{C}-\text{CH}_3\text{I}/^{11}\text{C}\text{-MeOTf}$ chemistry. Built by Peter Larsen of ScanSys (Copenhagen, Denmark) the rig has been installed at the cyclotron lab on campus, eliminating the need to drive $^{11}\text{C}\text{-CH}_4$ from the cyclotron to the Waisman Center for conversion to $^{11}\text{C}-\text{CH}_3\text{I}/^{11}\text{C}\text{-MeOTf}$. An evaluation of the performance of this chemistry box has been presented by Engle et al. (Engle et al 2008).

2.4 Radiosynthesis of $^{11}\text{C}$-FLB457

2.4.1 Labeling radiochemistry

Syntheses of $^{11}\text{C}$-FLB457 were performed via the intermediate $^{11}\text{C}\text{-MeOTf}$ using reaction conditions similar to those described previously (Lundkvist et al 1998). The precursor was obtained from ABX (Radeberg, Germany).

The precursor (FLB-604, 0.4 mg) was dissolved in 400 $\mu$L of acetone with 1 $\mu$L of 0.5 N sodium hydroxide (NaOH) added as base. In a stream of helium passing though a stainless steel needle, $^{11}\text{C}\text{-MeOTf}$ was bubbled through the precursor solution held at room temperature in a 1mL sealed V-vial. A vent needle coupled to a syringe packed with Porapak$^{\text{TM}}$ was inserted to the reaction vessel as to capture unreacted $^{11}\text{C}\text{-MeOTf}$. A radioactivity detector positioned for measurement of reaction vessel radioactivity was logged during the bubbling step, and when radioactivity reached a maximum, the needles were removed from the reaction vessel. Immediately following the bubbling step, the precursor solution was injected onto an HPLC for separation of precursor from product.
2.4.2 HPLC separation

Chromatographic separation was run via standard-phase HPLC at a pressure of about 1500 PSI (2.5 ml/min). Mobile phase is prepared by mixing 500 mL methylene chloride, 17 mL methanol and 70 µL triethylamine. Stationary phase consists of a 10 µm silica packed, 3.9x300mm, Waters μPorasil column (Part# WAT027477). A representative separation is shown in Figure 2-6. Retention time (\(t_r\)) for FLB457 is 90 seconds, and precursor is 125 seconds. The large UV peak preceding the FLB457 peak is acetone, the solvent used in the reaction mixture, which carries through unreacted \(^{11}\text{C}\)-MeOTf, both of which are volatile and evaporate during the roto-vap step following HPLC.
Figure 2-6: HPLC separation for $^{11}$C-FLB457 ($t_R = 90$ sec)

UV absorbance is shown by the solid line. Radioactivity detector is shown by the dotted line.

Following collection of the radiolabeled peak from the preparatory HPLC separation, the mobile phase was evaporated to dryness in a pear-vial using a roto-vap. The walls of the vessel were rinsed with 5 mL of a saline solution, taken up in a syringe, and passed through a 0.20 µm sterilization filter (Millex LG, Part# SLLG025SS) into an sterile, empty vial where it was ready for injection. Early syntheses utilized a Millex GS 0.22µm filter, but a large fraction of the product stuck to the filter, leading to the use of the Millex LG filter, which did not have the same problem.

2.4.3 Analytical HPLC

For measurement of the mass concentration of injected ligand, analytical HPLC was performed, as calibrated using reference standards. Mobile phase consisted of a 57 : 43 mixture of acetonitrile : 0.01 M
NaH$_2$PO$_4$ (0.3% HCl), run at 2.5 ml/min through a Nova-Pak C18 column (3.9x300mm, Waters part # 11695). Retention time was about 250 seconds, as measured by UV absorbance at 254 nm.

2.4.4 Results

 Decay corrected yields of the product following HPLC separation (as compared to $^{11}$C-MeOTf trapped in solution) range from 5-65%, with an average yield of about 30%. With starting activity ($^{11}$C-MeOTf) of over 100mCi, and a synthesis time of just over 10 minutes, these procedures often yielded 15-25 mCi of final product. The entire synthesis of $^{11}$C-FLB457 takes only about 22 minutes, from end of static bombardment to filtration of final product, as described below in Table 2-3.

**Table 2-3: Time course of $^{11}$C-FLB457 radiosynthesis**

<table>
<thead>
<tr>
<th>Time to End</th>
<th>Synthesis Step</th>
</tr>
</thead>
<tbody>
<tr>
<td>-22 min</td>
<td>Begin trapping methane on Hayesep</td>
</tr>
<tr>
<td>-17 min</td>
<td>Begin converting $^{11}$C-CH$_4$ to $^{11}$C-CH$_3$I</td>
</tr>
<tr>
<td>-10 min</td>
<td>Bubble $^{11}$C-MeOTf into precursor solution</td>
</tr>
<tr>
<td>-5 min</td>
<td>Load precursor solution onto HPLC</td>
</tr>
<tr>
<td>-3 min</td>
<td>Collect HPLC peak</td>
</tr>
<tr>
<td>-2 min</td>
<td>Evaporate HPLC solvents</td>
</tr>
<tr>
<td>-1 min</td>
<td>Reconstitute and filter</td>
</tr>
<tr>
<td>0 min</td>
<td>Radiotracer ready for injection</td>
</tr>
</tbody>
</table>
2.5 Radiosynthesis of $^{11}$C-fallypride

2.5.1 Labeling radiochemistry

Initial syntheses of $^{11}$C-fallypride were performed via the intermediate $^{11}$C-CH$_3$I using reaction conditions as described previously using the precursor [benzamide, 5-(3-fluoropropyl)-2,3-dimethoxy-N-[(2S)-1-(2-propenyl)-2-pyrrolidinyl]methyl]-, CAS RN 166173-78-0] (Mukherjee et al 2004). To obtain higher radiochemical yields, the reaction was modified as to use the more reactive $^{11}$C-MeOTf (Murali et al. 2009). The precursor was obtained from Jogesh Mukherjee of the University of California - Irvine.

2.5.1.1 $^{11}$C-CH$_3$I reaction conditions

The phenolic precursor (1.0 mg) is dissolved in 100 µL of dimethylformamide (DMF), with 1 µL tetrabutylammonium hydroxide (TBAH) added as base, as described previously (Mukherjee et al 2004). In a stream of helium passing though a stainless steel needle, $^{11}$C-CH$_3$I was bubbled through the precursor solution in a 1mL sealed vessel. As described in the section with $^{11}$C-FLB457, a radioactivity detector’s reading is used for determination of when to stop the bubbling step. Following the trapping of $^{11}$C-CH$_3$I in solution, and allowing time to react, the product and precursor were separated via HPLC. The reaction scheme for labeling $^{11}$C-fallypride via $^{11}$C-CH$_3$I is shown below in Figure 2-7.
The phenolic precursor (0.5 mg) is dissolved in 300 µL of anhydrous acetonitrile (ACN), with 10 µL 5N sodium hydroxide (NaOH) added as base. In a stream of helium passing through a stainless steel needle, $^{11}$C-MeOTf was bubbled through the precursor solution held at room temperature in a 5mL sealed Wheaton V-vial containing a miniature stir bar. Following bubbling, the sealed reaction vessel was then heated at 80°C for 5 minutes before adding 0.7mL of mobile phase to the reaction vessel, quenching the reaction. The reaction scheme for labeling $^{11}$C-fallypride via $^{11}$C-MeOTf is shown below in Figure 2-8.

**Figure 2-7:** Radiosynthesis of $^{11}$C-fallypride via $^{11}$C-CH$_3$I

2.5.1.2 $^{11}$C-MeOTf reaction conditions

The phenolic precursor (0.5 mg) is dissolved in 300 µL of anhydrous acetonitrile (ACN), with 10 µL 5N sodium hydroxide (NaOH) added as base. In a stream of helium passing through a stainless steel needle, $^{11}$C-MeOTf was bubbled through the precursor solution held at room temperature in a 5mL sealed Wheaton V-vial containing a miniature stir bar. Following bubbling, the sealed reaction vessel was then heated at 80°C for 5 minutes before adding 0.7mL of mobile phase to the reaction vessel, quenching the reaction. The reaction scheme for labeling $^{11}$C-fallypride via $^{11}$C-MeOTf is shown below in Figure 2-8.
2.5.2 HPLC separation

Chromatographic separation of $^{11}$C-fallypride from its precursor was performed via reverse-phase HPLC, with a flow rate of 5 ml/min. Mobile phase consisted of a 56:44:0.1 mixture of acetonitrile/water/triethylamine. Stationary phase consisted of a C18 6µm resin in a Waters Nova-Pak HR column (Part# WAT025820, 7.8x300mm). A representative separation is shown below in Figure 2-9. Retention time of fallypride is 400 seconds, and precursor is about 100 seconds.
Following collection of the radiolabeled HPLC peak into a pear-vial, the mobile phase was evaporated to dryness using a roto-vap. The walls of the vessel were rinsed with 5 mL of a saline solution and passed through a 0.20 µm sterilization filter (Millex LG, Part# SLLG025SS) and into an evacuated sterile vial where it was ready for injection.

2.5.3 Analytical HPLC

For measurement of the mass concentration of injected ligand, analytical HPLC was performed, as calibrated using reference standards. Mobile phase consisted of a 57 : 43 mixture of acetonitrile : 0.01 M NaH₂PO₄ (0.3% HCl), run at 2.5 ml/min through a Nova-Pak C18 column (3.9x300mm, Waters part # 11695). As measured by UV absorbance at λ = 254 nm, retention time was about 410 seconds.
2.5.4 $^{11}$C-fallypride reaction results

Decay corrected yields (product following roto-vap, as compared to $^{11}$C-MeOTf/$^{11}$C-CH$_3$I trapped in solution) improved from a range from 0.4-3.1% when using $^{11}$C-CH$_3$I to an average of 59% ± 10% when using $^{11}$C-MeOTf. These procedures often yielded 30-50 mCi of final product with starting activity ($^{11}$C-MeOTf) of over 150mCi.

The synthesis of $^{11}$C-fallypride takes about 37 minutes following end of bombardment, as described below in Table 2-4. Compared to $^{11}$C-FLB457, the additional 15 minutes can be attributed to the 5 minute cooking step, a longer HPLC retention time, and more time needed for evaporation of HPLC mobile phase. The additional synthesis time of $^{11}$C-fallypride limits the maximum specific activity of this tracer to about 50% of that achievable for $^{11}$C-FLB457. In an experiment aimed at reducing the synthesis time, one synthesis was performed without the 5 minute heating step, but this modification was abandoned due to a very low radiochemical yield, thus justifying the use of the 5 minute cooking step.
2.6 Radiosynthesis of $^{18}$F-fallypride

2.6.1 Labeling radiochemistry

After proton irradiation (>5 µA, >45 minutes) of a pressurized water target, the target water removed from the target and passed through a QMA anion-exchange column pre-rinsed with a potassium bicarbonate/water solution, trapping $^{18}$F-F$^{-}$ ions. The $^{18}$F-F$^{-}$ ions were then eluted from the column with a solution of 1ml Kryptofix 2.2.2 and 0.5 mL acetonitrile into a Wheaton V-vial to be loaded into the automated chemistry box.

Chemical synthesis of $^{18}$F-fallypride was carried out in a modified chemistry process control unit (CPCU) (CTI, Knoxville, TN), providing an automated method for nucleophilic substitution of $^{18}$F-F$^{-}$ with
fallypride’s tosyl precursor (ABX, Radeberg, Germany) using a method similar to that described previously (Mukherjee et al 1995). The reaction scheme is shown below in Figure 2-10.

Figure 2-10: Reaction scheme for $^{18}$F-fallypride

The Kryptofix/$^{18}$F- solution is evaporated to dryness in the CPCU reaction vessel in an 120°C oil bath while bubbling a stream of argon through the solution. After evaporation, a precursor solution consisting of 2 mg precursor dissolved in 0.8 ml of acetonitrile was added to the reaction vessel and allowed to react at 87°C for 12 minutes, during which the evaporated solvent (acetonitrile) was replaced every 3 minutes. Following the reaction step, the contents of the reaction vessel are mixed with 1.2 ml ethanol three times, with each rinse being passed through an ethanol-rinsed (10ml) alumina N cartridge (Waters, WAT023501) as to remove unreacted $^{18}$F-F$^{-}$ from the mixture. The ethanol-based solution is then evaporated to dryness at 120°C. The drying vessel is then rinsed with 1.5 mL of HPLC mobile phase and passed out of the CPCU for injection onto the HPLC injection loop.
2.6.2 HPLC separation

Chromatographic separation was run via reverse-phase HPLC, run at 2.5 ml/min. Mobile phase consisted of a 25:75 mixture of ethanol an 0.01 M solution of sodium dihydrogen phosphate (0.3% HCl), pumped through a Waters µBondapak CN 10µm column (WAT84177, 7.8x300). A representative separation is shown in Figure 2-11. Retention time of fallypride is 770 seconds.

The radiolabeled peak is collected manually, with a volume of ~2.5mL. This is diluted with 10 mL of saline, and passed through a 0.20 µm sterilization filter (Millex LG, Part# SLLG025SS) and into a evacuated sterile vial.

![HPLC trace for ^18F-fallypride](image)

*Figure 2-11: HPLC trace for ^18F-fallypride*
2.6.3 Analytical HPLC

For measurement of the mass concentration of injected ligand, analytical HPLC was performed, as calibrated with a reference standard. Mobile phase consisted of a 65 : 35 mixture of acetonitrile : 0.01 M NaH₂PO₄ (0.3% HCl), run at 2.0 ml/min through a Spherisorb ODS2 5µ column (4.6x250mm, Waters part # PSS 831915). As measured by UV absorbance at $\lambda = 254$ nm, retention time is about 16 minutes.

2.6.4 Results

Decay corrected yields of (collected product activity as compared to starting $^{18}$F-F$^-$ activity) range from 5-25%, with an average yield of about 15%. With starting activity ($^{18}$F-F$^-$) of over 300mCi, and a synthesis of about 90 minutes, these procedures often yield 15-25 mCi of final product.
2.7 References

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Chapter 3

In Vivo Characterization of $^{18}$F-Fallypride and $^{11}$C-FLB457

3.1 Introduction

This chapter describes a set of five PET experiments performed using the multiple-injection technique, used to provide full characterization of the \textit{in vivo} properties of both $^{18}$F-fallypride and $^{11}$C-FLB457. This type of experiment is important for these PET radioligands provide the ability to image receptors in low concentrations (<1 pmol/mL, Figure 3-1), and serve as valuable biomarkers for studying the dopamine system. A number of studies have been performed to characterize the \textit{in vivo} kinetics of these radiotracers and to evaluate experimental designs for measuring extrastriatal D$_2$D$_3$ receptor binding.

For the assessment of endogenous dopamine competition, it has been suggested that the radiotracer vascular transport rate constants, $K_1$ and $k_2$, have a significant effect on the sensitivity for detecting endogenous dopamine release (Morris and Yoder 2006), despite these processes not being directly related to receptor binding. In a challenge-type experiment (i.e. amphetamine challenge), where competitive dopamine release is evoked after the administration of radioligand, it was simulated that $^{11}$C-raclopride yielded the highest sensitivity for detecting DA release in the striatum. This comparison was performed with a variety of radiotracers including $^{18}$F-fallypride, $^{11}$C-FLB457. The increased detection sensitivity
was not attributed to the rate constants involved in specific binding of the radioligand to the receptor site, but rather to the tissue to plasma efflux rate constant, $k_2$. A fast $k_2$ constant provides rapid clearance of the radioligand from the free space following displacement by endogenous neurotransmitter, thus enhancing the change in the PET signal which represents both bound and free states. However, in the case of extrastriatal D2/D3 receptor binding, $^{11}$C-raclopride does not have sufficient target to background signal to detect subtle changes in the specific binding. In these regions, higher affinity radiotracers are required to provide a suitable signal from the specifically bound component of the PET measurement.

High affinity by itself is not sufficient for extrastriatal assay, a radiotracer must also have low nonspecific uptake, which is dependent on lipophilicity and nonspecific protein binding. This issue was illustrated by a semi-quantitative in vivo comparison of $^{11}$C-fallypride, $^{11}$C-FLB457 with $^{11}$C-cyclopropyl-FLB457 (Airaksinen et al 2006). $^{11}$C-cyclopropyl-FLB457 has been investigated as a candidate radioligand for extrastriatal D2/D3 binding due to its high in vitro D2/D3 affinity, which is 10-fold greater than fallypride and FLB457 (Airaksinen et al 2008). Semiquantitative comparisons of these radiotracers revealed similar target/cerebellum ratios in the subcortical and cortical regions, despite the significantly higher affinity of $^{11}$C-cyclopropyl-FLB457 for D2/D3 receptors. Though not experimentally confirmed, these findings suggest a higher nonspecifically bound component for $^{11}$C-cyclopropyl-FLB457 in the cerebellum.

Designing and conducting PET experiments to separate and uniquely identify the radioligand delivery and binding components is challenging for a single bolus injection PET study. Frequently there is a high covariance between the parameter estimates of delivery ($K_1, k_2$) and binding ($k_3, k_4$), particularly for high affinity radiotracers with rapid specific binding ($k_3$). This high covariance limits the interpretation of each parameter independently. To effectively uncouple the in vivo parameters for the characterization of $^{18}$F-
fallypride and $^{11}$C-FLB457, it is necessary to introduce several injections of ligand, each time varying the concentrations of unlabeled and radiolabeled ligand. These multiple-injection (MI) PET experiments methodologically perturb and observe the system to separate the high covariance between parameters by introducing competition between the labeled and unlabeled ligand for the receptor site (Morris et al. 2004).

MI PET strategies have been employed for both FLB457 and fallypride to provide in vivo estimation of radioligand-receptor binding characteristics. Using the long-lived $^{76}$Br ($t_{1/2} = 16.1$ hr) radiolabel and the MI technique, FLB457 has been evaluated in baboons for the estimation of receptor density ($B_{\text{max}}$) (Delforge et al. 1999) and for assay of endogenous dopamine competition (Delforge et al. 2001). In humans, MI studies of $^{11}$C-FLB457 have been reported to assess the effects of unlabeled ligand competition (Olsson et al. 2004) and the use of the cerebellum as a reference region (Asselin et al. 2007a). For $^{18}$F-fallypride, MI studies have been reported in rhesus monkeys for in vivo characterization (Christian et al. 2004) and in baboons for the measurement of the in vivo affinity (Slifstein et al. 2004a). It must be stressed that MI studies are uniquely designed to optimize the estimation of a particular parameter of interest. For example, an MI design occurring over several separate imaging sessions can yield estimates of the apparent affinity (via scatchard type of analysis) (Holden et al. 2002) but are often not suitable for uncoupling the radioligand transport parameters ($K_1,K_2$), which is possible with a single-session MI study. Thus, attempting to compare the in vivo characteristics of FLB457 and fallypride based upon the literature findings is difficult because the experiments were not optimized for direct comparison.

The goal of the experiments described in this chapter was to perform a direct comparison of $^{18}$F-fallypride and $^{11}$C-FLB457 using the multiple-injection protocol in the rhesus monkey model. The experiments were designed to obtain estimates of both the radioligand transport and binding parameters, with
particular interest in the tissue-to-plasma efflux constant \(k_2\) and the *in vivo* equilibrium dissociation constant \(K_{\text{Dapp}}\). Knowledge of these radioligand characteristics will aid in the design of future experiments with the goal of maximizing sensitivity to subtle differences in extrastriatal \(D_2/D_3\) receptor binding and endogenous dopamine competition in applications for studying diseases that where disruptions in the dopaminergic system are implicated.

### 3.2 Experimental procedures

#### 3.2.1 Optimization of experimental design

Values based on the previous multiple-injection studies (Christian *et al* 2004; Delforge *et al* 1999) were used as a staring point to a multiple-step optimization procedure. The first step was to use analytical sensitivity analysis to find a number of protocols that could be suited for providing good identifiability of all parameters. Next, these protocols were evaluated using Monte Carlo methods where many instances of simulated PET data were fit for each protocol from the sensitivity analysis. The protocol giving the most accurate and precise estimation of the known true values was chosen. This whole process was repeated before each PET scanning session, using refined starting estimates based on acquired data.

*Details*

A single session, multiple-injection (MI) design was chosen for this protocol utilizing the hot-cold model to describe the *in vivo* kinetics of the ligands (Delforge *et al* 1990). Initial designs investigated protocols with 3 serial injections of radiolabeled and unlabeled ligand. The entire experimental design was constrained to be less than 3 hours. The MI experiments were selected with the goal of yielding optimal parameter identifiability for the PET model parameters describing plasma–tissue transport \([K_1, k_2]\) and
receptor ligand-receptor binding $[f_{ND}, k_{on}, k_{off}, B_{max}]$ (Innis et al 2007), with special focus given to regions of low binding, such as the cortex.

Sensitivity analysis was performed to determine the injection protocol that maximizes the determinant of the reduced hessian matrix ($H_R$) based upon the preliminary parameter estimates reported for fallypride (Christian et al 2004) and FLB457 (Delforge et al 1999) and using arterial plasma input functions obtained from preliminary studies. Only the binding parameters ($f_{ND}, k_{on}, B_{max}, k_{off}$) were included in $H_R$. Parameters for the optimization algorithms included injection times for the second and third injections as well as the activity and mass injected for each of the three injections. The optimization was performed to select the parameters that yielded the highest parameter precision in the $H_R$.

Monte Carlo simulations were then performed to assess the effects of noise on parameter precision and to examine the sensitivity for the estimation of $K_1$ and $k_2$, which were not considered in the preliminary experimental design. These simulations were performed by simulating a MI PET curve, adding noise to the simulated data, and fitting the noisy simulated data using methods as described below.

**Implementation and protocol revisions**

The experimental protocols used for the multiple injection studies are given in Table 3-1. Following the initial $^{18}$F-fallypride study (M1), the optimization protocol was revised with a new set of parameter estimates. The second $^{18}$F-fallypride study (M2) used a revised protocol with only two injections. After the first $^{11}$C-FLB457 study, transport ($K_1, k_2$) and $k_{off}$ estimates were updated, and optimization procedures
gave a new protocol for M2, increasing the time between injections. After acquisition of the second $^{11}$C-FLB457 study, the protocol was revised again and used as a second $^{11}$C-FLB457 experiment in M1.

### 3.2.2 Preparation of $^{18}$F-fallypride and $^{11}$C-FLB457

The details of radiochemistry procedures is given in Chapter 2. For each study, a stock solution of either unlabeled FLB457 or unlabeled fallypride was prepared by dissolving a reference standard in a sterile 10% ethanol/saline solution. For the second and third injections of each experiment, a given volume of the stock solution was thoroughly mixed with the labeled radiotracer, lowering its specific activity as needed per the scan injection protocol.

### 3.2.3 PET scans

MI PET studies were acquired on 2 male rhesus monkeys (*macaca mulatta*; M1: 7 kg, 6 years; M2: 8 kg, 4 years) using the experimental protocols described in Table 3-1. Experimental procedures were approved by the University of Wisconsin Institutional Animal Care and Use Committee. For the scanning procedure, the monkey was initially anesthetized with ketamine (10mg/kg i.m.) and maintained with 0.75-1.5% isoflurane for the duration of the scan. Atropine was given at 0.25 mg/animal (i.m.) to minimize secretions. Body temperature, breathing rate, heart rate and SpO$_2$ levels were monitored and logged during the course of each scanning session. A catheter was placed in the saphenous vein for the administration of ligand and another catheter was placed in the femoral artery for withdrawing arterial plasma samples.
### Table 3-1: Experimental Protocols

<table>
<thead>
<tr>
<th>Animal/experiment</th>
<th>M1a</th>
<th>M2a</th>
<th>M1b</th>
<th>M2b</th>
<th>M1c</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tracer</strong></td>
<td>¹⁸F-fallypride</td>
<td>¹⁸F-fallypride</td>
<td>¹¹C-FLB457</td>
<td>¹¹C-FLB457</td>
<td>¹¹C-FLB457</td>
</tr>
<tr>
<td><strong>t₁</strong> (min)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>a₁</strong> (mCi)</td>
<td>3.57</td>
<td>3.20</td>
<td>3.29</td>
<td>3.32</td>
<td>1.59</td>
</tr>
<tr>
<td><strong>m₁</strong> (nmol)</td>
<td>2.0</td>
<td>0.7</td>
<td>1.8</td>
<td>0.7</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>SA₁</strong> mCi/µmol</td>
<td>1,800</td>
<td>4,500</td>
<td>1,800</td>
<td>4,800</td>
<td>1,600</td>
</tr>
<tr>
<td><strong>t₂</strong> (min)</td>
<td>100</td>
<td>100</td>
<td>30</td>
<td>40</td>
<td>100</td>
</tr>
<tr>
<td><strong>a₂</strong> (mCi)</td>
<td>3.3</td>
<td>3.0</td>
<td>3.4</td>
<td>3.4</td>
<td>3.6</td>
</tr>
<tr>
<td><strong>m₂</strong> (nmol)</td>
<td>13.1</td>
<td>10.6</td>
<td>11.6</td>
<td>12.8</td>
<td>11.4</td>
</tr>
<tr>
<td><strong>SA₂</strong> mCi/µmol</td>
<td>250</td>
<td>280</td>
<td>290</td>
<td>260</td>
<td>310</td>
</tr>
<tr>
<td><strong>t₃</strong> (min)</td>
<td>150</td>
<td>--</td>
<td>60</td>
<td>81</td>
<td>150</td>
</tr>
<tr>
<td><strong>a₃</strong> (mCi)</td>
<td>1.27</td>
<td>--</td>
<td>0.33</td>
<td>0.51</td>
<td>3.17</td>
</tr>
<tr>
<td><strong>m₃</strong> (nmol)</td>
<td>100</td>
<td>--</td>
<td>109</td>
<td>102</td>
<td>111</td>
</tr>
<tr>
<td><strong>SA₃</strong> mCi/µmol</td>
<td>13</td>
<td>--</td>
<td>3</td>
<td>5</td>
<td>29</td>
</tr>
<tr>
<td><strong>t₄</strong> (min)</td>
<td>180</td>
<td>150</td>
<td>120</td>
<td>121</td>
<td>182</td>
</tr>
</tbody>
</table>
The PET scans were acquired on a Concorde microPET P4 scanner (Tai et al. 2001) with the animal mounted in a custom head holder in the prone position. Following positioning, attenuation scans were acquired for 518 seconds using a $^{57}$Co transmission point source. Collection of emission data was initiated with the first bolus injection of radiotracer. Following the scan, the animal was removed from the scanner bed and from anesthesia. Upon recovery of the swallowing reflex, the animal was returned to its home cage where it was monitored until it was alert.

### 3.2.4 Image processing

The PET list mode data were binned into sinograms with durations of 30 seconds per frame with corrections applied for scanner deadtime and random coincidence events. Emission sinograms were reconstructed with filtered backprojection using a 0.5cm$^{-1}$ ramp filter, zoom of 1.5, and 128x128x63 voxel matrix size with voxel size of 1.26mm x 1.26mm x 1.21mm. Corrections applied for attenuation, scanner normalization, and scatter to create images with quantitative units of nCi/cc. The data were not decay corrected, as radioactive decay is accounted for in the hot-cold model (see section 3.2.6).

The reconstructed time series images for each monkey were spatially transformed into a common space for the generation of regional time-activity curves. A rigid body registration was performed to the integrated images of $^{11}$C-FLB457 and $^{18}$F-fallypride using the FSL flirt software (Smith et al. 2004) and the affine transformation was applied to the entire dynamic PET series. Regions of interest (ROIs) were manually placed on each image slice within the boundaries of each region, as visualized on an the template image for each monkey. Time-activity curves represent average radioactivity concentration within each ROI for each frame. The ROI placement for each region is shown in Figure 3-2. The thalamus region (0.13cm$^3$) was placed predominantly over the medial dorsal region of the thalamus, the
substantia nigra (SN) (0.14cm³) over the pars compacta and other SN sub-regions unidentifiable in PET images, the temporal cortex region (0.87cm³) represents the superior temporal sulcus, and the frontal cortex region (1.26cm³) encompasses the dorsal prefrontal cortex. No distinction was made between left and right regions. The cerebellum ROI (0.63cm³) was drawn over the cortex of the cerebellar lobes and positioned to avoid signal from the white matter, the vermis and the occipital cortex.

![Image of ROIs](image-url)

**Figure 3-2: ROI locations**

Representative locations of ROIs as shown on a single slice. While shown on a single slice, the ROIs were drawn on multiple adjacent slices in the 3D volume image space.

### 3.2.5 Input function determination

Arterial blood samples (~0.5ml each) were drawn throughout the course of each study. Sampling frequency varied from 10 samples/minute following each injection to 0.1 samples/minute after 30 minutes post injection. The whole blood samples were mixed with heparinized saline and assayed for radioactivity using a 3” NaI(Tl) well counter, cross-calibrated to the PET scanner to give measurements in nCi. Blood samples were then centrifuged at 2200xG for 5 minutes and 250µL plasma was extracted. The plasma samples were alkynized with 50µL sodium bicarbonate before undergoing two ethyl acetate
extractions (500µL each) to extract the lipophilic species. Both extractions were combined and assayed and converted to units of nCi/mL after applying a correction for extraction efficiency (~90%). The ethyl acetate was evaporated and thin-layer-chromatography was performed to assess the fraction of lipophilic plasma that could be attributed to lipophilic metabolites.

The whole blood and parent ligand plasma time-activity curves were then parameterized to an analytic function with 3 exponents to describe the decline of the radiotracer, as shown in Figure 3-3. Curve stripping was used to generate separate time courses corresponding to each injection subject to a constraint that the slowest component of decline was equal for all injections of each study. The parent ligand plasma time-activity data were converted to units of molar concentration (pmol/mL) by division by the injected specific activity. Decay correction was applied to the molar concentration to represent the “cold” ligand, which does not undergo radioactive decay.
Figure 3-3: Model input function shape

Each component of the input function shape is described analytically with the set of equations shown.

### 3.2.6 Multiple injection model

**Model Description**

The differential equations for the multiple injection model describing the rate of change of the ligand concentration in the free, $F_i$; and bound, $B_i$; compartments (for each injection $i$) are given in the following equations:

$$\frac{dF_i}{dt} = K_i C_{p_i} - k_{f} F_i - f_{ND} k_{on} \left( B_{max} - \sum_i B_i \right) B_i + k_{off} B_i$$

$$\frac{dB_i}{dt} = f_{ND} k_{on} \left( B_{max} - \sum_i B_i \right) B_i - (k_{off}) B_i$$
For 3-injection experiments, there are a total of three differential equations describing the time rate of change of the free ligand concentration \( (dF/dt) \) and another three differential equations describing bound ligand concentration \( (dB/dt) \). These six differential equations use the same values for the plasma to tissue rate constant \( (K_1) \), tissue to plasma efflux constant \( (k_2) \), the ligand – receptor association \( (f_{NDk_{on}}) \), dissociation \( (k_{off}) \) rate constants and the receptor density \( (B_{max}) \). The molar concentration of the ligand in the arterial plasma \( (C_{pi}) \) for each injection serves as the input function to the model. The output signal measured by the PET scanner \( (Model_j) \) is related to the state variables by the equation:

\[
Model_j = \frac{1}{t_{end} - t_{begin}} \int_{t_{begin}}^{t_{end}} \left\{ \sum_i [SA_i(t)(1 - F_i)(F_i(t) + B_i(t))] + F_i C_{WB}(t) \right\} dt
\]

with \( J \) dynamic PET frames and specific activity injection of \( SA_i \) for each injection. The bloodborne component in each region is modeled as the whole blood time course \( (C_{WB}(t)) \) multiplied by the fractional blood volume \( (F_V) \), which was set at \( F_V = 0.04 \).

Model configuration and parameter estimation was performed using the COMKAT software algorithms (Muzic and Cornelius 2001). A constrained non-linear search using the Levenberg-Marquart optimization algorithm was performed for estimation of the kinetic parameters.

Parameter estimates were made both on an individual ROI basis and simultaneously in multiple regions using an objective function that minimized the total sum of squares residual, \( RSS \), across \( N \) ROIs consisting of \( J \) frames each, as given below.

\[
RSS(p) = \sum_{n=1}^{N} \sum_{j=1}^{J} w_{n,j} \left( PET_{n,j} - Model_{n,j} \right)^2
\]
Depending on what parameters we were trying to estimate, a subset of the $p$ was used, where $p = [f_{ND}k_{on}, k_{off}, [K_{1}, k_{2}, B_{max}]]$. Uniform weighting $w_{n,j}$ was used for all of the frames, which were of equal duration. All rate constants $[K_{1}, k_{2}, f_{ND}k_{on}, k_{off}]$ were constrained to fall within the bounds of $[0,1]$ (min$^{-1}$, ml·cm$^{-3}$·min$^{-1}$). $B_{max}$ was constrained to $[0,100]$ (nmol·L$^{-1}$).

To increase parameter identifiability, estimation was performed in a stepwise fashion. We first determined $k_{off}$, then $K_{1}, k_{2}, f_{ND}k_{on}$, and $B_{max}$. Finally transport parameters and receptor concentration was determined in the cerebellum. The procedures described herein follow the assumption that all rate constants and concentrations of receptor density remain constant throughout the duration of the scanning session.

**Determination of $k_{off}$**

First, $k_{off}$ was estimated on an individual ROI basis in the medium and high density regions (caudate, thalamus, and substantia nigra). These initial estimates of $k_{off}$ used $p = [f_{ND}k_{on}, k_{off}, K_{1}, k_{2}, B_{max}]$ with $n = 1$. Based on the results of the initial fit, we modified our fitting procedure to estimate a single $k_{off}$ value for all regions, where $p = [f_{ND}k_{on}, k_{off}, [K_{1}, k_{2}, B_{max}]]$ and $n = 1, 2, 3$. These methods provided a single $k_{off}$ value for each individual study.
Estimation of $K_1$, $k_2$, $f_{ND}k_{on}$, and $B_{max}$

A similar, multi-step strategy was used for the estimation of $K_1$, $k_2$, $f_{ND}k_{on}$, and $B_{max}$. Initial estimates of these parameters used four ROIs ($n = 1,2,3,4$): thalamus, SN, PFC, and TmpCtx. This initial estimate used a fixed value for $k_{off}$ as determined earlier. For M1 FLB457 studies, the $k_{off}$ determined from the first study was applied to the second FLB457 study. This optimization used $p = \left[f_{ND}k_{on}, [K_{1n}, k_{2n}, B_{max}]_n \right]$. For our second iteration of parameter estimation, we fixed values of $B_{max}$, using $p = \left[f_{ND}k_{on}, [K_{1n}, k_{2n}]_n \right]$. For M1, the fixed $B_{max}$ values were the average of the $B_{max}$ values found in $^{11}$C-FLB457 and $^{18}$F-fallypride studies. For M2, the fixed $B_{max}$ values were those determined by the $^{18}$F-fallypride study. The apparent equilibrium dissociation constant, $K_{Dapp} = k_{off}/f_{ND}k_{on}$, was calculated for each monkey.

Fitting cerebellum data

In the cerebellum, the rate constants were determined using both a one-compartment model (1CM) and a two-compartment model (2CM). For 1CM fitting, we used $p = [K_1, k_2]$, and for 2CM fitting we used $p = [K_1, k_2, B_{max}]$ with $f_{ND}k_{on}$ and $k_{off}$ fixed to values determined using the methods described above. Akaike information criterion (Akaike 1974) was calculated for comparing the models to examine if the presence of the additional term for receptor density ($B_{max}$) in the two-compartment model was justified.
3.3 Results

3.3.1 Optimization of experimental design

Using Monte Carlo methods, it was found that the identifiability of $K_1$ and $k_2$ was not affected by the experimental design within the range of schemes required for $f_{NDk_{on}}$, $k_{off}$ and $B_{max}$ identifiability. For $^{18}$F-fallypride, it was found that parameter identifiability could be achieved with only two injections, thus the saturating dose (injection 3) was eliminated for the fallypride protocol for M2. For $^{11}$C-FLB457, initial parameter estimates used for optimization led to a protocol with injection times at 0, 30 and 60 minutes (see Table 3-1). This preliminary study did not adequately identify $B_{max}$ and $f_{NDk_{on}}$, yielding a correlation between parameter estimates greater than 0.9. This protocol was then refined for M2 increasing the amount of time between the injections, but $f_{NDk_{on}}$ and $B_{max}$ parameters could still not be identified. The third experiment with $^{11}$C-FLB457 introduced even longer delays between injections, leading to better identifiability of $f_{NDk_{on}}$ and $B_{max}$.

3.3.2 Input function determination

The results of the input function fitting procedure for the M1 $^{18}$F-fallypride study are shown below in Figure 3-4. Fits to other input functions provide similar results.
Figure 3-4: Fit of model to measured plasma data

(A) Measured input function radioactivity from the M1 fallypride study (circles) and the fit to measured data, fit with three separate input functions (dotted lines, IF #1-3) and the sum of the three input functions (solid line). (B) Three input functions scaled by specific activity, shown in units of pmol/cc.

Figure 3-5 shows a comparison of the arterial plasma time-activity curves of parent radioligand for $^{11}$C-FLB457 and $^{18}$F-fallypride. The data are normalized to the injected dose and shown for the first 40 minutes post-injection and averaged over the three injections. These plots show that the native $^{11}$C-FLB457 was cleared from the arterial plasma significantly faster than the native fallypride. The fraction of parent compound was 2 – 4 times higher for $^{18}$F-fallypride than for $^{11}$C-FLB457 at roughly 5 minutes post-injection. The faster rate of clearance of $^{11}$C-FLB457 continued throughout the course of the study, with the slowest exponential component of 0.033 min$^{-1}$ for $^{11}$C-FLB457 and 0.017 min$^{-1}$ for $^{18}$F-fallypride, on average. The rapid metabolism for both tracers resulted in hydrophilic species which did not cross the blood brain barrier. At later time points the lipophilic metabolites were present in the ethyl acetate extraction along with the parent compound. Because the protocol used herein involved multiple
injections of radioligand, the presence of the lipophilic fraction had a negligible effect on the parameter estimates, for the relative proportion remained small with the addition of parent compound at each injection.

**Figure 3-5:** Comparison of parent plasma input curves

Fraction of the injected dose, time shifted to t= 0. Each curve represents the average of all injections of a particular study. Dotted lines are for fallypride, solid lines are for FLB457.

### 3.3.3 Parameter estimation

**Determination of $k_{\text{off}}$**

For parameter estimation of the specific binding parameters, identifiability was best for $k_{\text{off}}$. $k_{\text{off}}$ displayed a small covariance with the other parameters and was found to be consistent ( < 30% variability ) across regions when determined separately for the caudate, SN, and thalamus. The values of $k_{\text{off}}$ for each study, as constrained to take on a single value across regions for a given experiment, are given in the top row of
Table 3-2. These procedures show that $k_{off}$ is slower for $^{11}$C-FLB457 than for $^{18}$F-fallypride, with an average value of 0.016 min$^{-1}$ and 0.022 min$^{-1}$, respectively.

**Estimation of $K_D$, $k_2$, $f_{ND}k_{on}$, and $B_{max}$**

The parameter estimates for $^{18}$F-fallypride and $^{11}$C-FLB457 for M1 and M2 are given in Table 3-2. Figure 3-6A shows an example of the measured PET data and the model’s fit to the data.

All of the experiments for both $^{11}$C-FLB457 and $^{18}$F-fallypride demonstrated high identifiability for both the transport parameters and the ligand-receptor interaction parameters, with the exception of $f_{ND}k_{on}$ for $^{11}$C-FLB457 for M2. Ranges given for M2 for $f_{ND}k_{on}$ and $k_{off}$ represent the mid-range of values that provided similarly acceptable fits to the data. When averaged across both monkeys, $K_D$ for $^{18}$F-fallypride is higher than that of $^{11}$C-FLB457 (0.39 nM vs 0.13 nM, respectively).

**Fitting cerebellum data**

Parameter estimates for cerebellar data are shown in Table 3-2. An example of the data fit for M1 is shown in Figure 3-6B, comparing both two compartment (2CM) and one compartment (1CM) models. For both $^{11}$C-FLB457 studies, the two-compartmental model provided the most appropriate fit using the Akaike information criterion, which is visually evident in Figure 3-6B. For $^{18}$F-fallypride, the one-compartment model was adequate for describing the data. It was also found that $^{11}$C-FLB457 displayed considerably higher volume of distribution, $V_{ND}$ (= $K_1/k_2$), primarily attributed to a lower $k_2$. This difference is consistent for both the 1CM or 2CM model.
Table 3-2: Results of fitting procedures

<table>
<thead>
<tr>
<th></th>
<th>M1 (18F-fallypride)</th>
<th>M2 (11C-FLB457)</th>
<th>M1 (18F-fallypride)</th>
<th>M2 (11C-FLB457)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( f_{ND}K_{on} ) nmol·L(^{-1})·min(^{-1} )</td>
<td>0.046</td>
<td>0.13</td>
<td>0.080</td>
<td>0.15**</td>
</tr>
<tr>
<td>( k_{off} ) min(^{-1} )</td>
<td>0.024</td>
<td>0.014</td>
<td>0.021</td>
<td>0.018**</td>
</tr>
<tr>
<td>( k_{Dapp}^* ) nmol·L(^{-1} )</td>
<td>0.52</td>
<td>0.11</td>
<td>0.25</td>
<td>0.15**</td>
</tr>
<tr>
<td>PFC ( K_1 ) ml·cm(^{-3})·min(^{-1} )</td>
<td>0.22</td>
<td>0.49</td>
<td>0.55</td>
<td>0.53</td>
</tr>
<tr>
<td>( k_2 ) min(^{-1} )</td>
<td>0.37</td>
<td>0.13</td>
<td>0.46</td>
<td>0.20</td>
</tr>
<tr>
<td>( B_{max} ) nmol·L(^{-1} )</td>
<td>0.6</td>
<td>0.4</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>THalamus ( K_1 ) ml·cm(^{-3})·min(^{-1} )</td>
<td>0.42</td>
<td>0.66</td>
<td>0.76</td>
<td>0.66</td>
</tr>
<tr>
<td>( k_2 ) min(^{-1} )</td>
<td>0.49</td>
<td>0.18</td>
<td>0.68</td>
<td>0.25</td>
</tr>
<tr>
<td>( B_{max} ) nmol·L(^{-1} )</td>
<td>1.5</td>
<td>0.8</td>
<td>1.5</td>
<td>0.8</td>
</tr>
<tr>
<td>SN ( K_1 ) ml·cm(^{-3})·min(^{-1} )</td>
<td>0.35</td>
<td>0.36</td>
<td>0.41</td>
<td>0.40</td>
</tr>
<tr>
<td>( k_2 ) min(^{-1} )</td>
<td>0.54</td>
<td>0.10</td>
<td>0.53</td>
<td>0.19</td>
</tr>
<tr>
<td>( B_{max} ) nmol·L(^{-1} )</td>
<td>3.0</td>
<td>2.3</td>
<td>3.0</td>
<td>2.3</td>
</tr>
<tr>
<td>Cbm (2CM) ( K_1 ) ml·cm(^{-3})·min(^{-1} )</td>
<td>0.57</td>
<td>0.65</td>
<td>0.51</td>
<td>0.53</td>
</tr>
<tr>
<td>( k_2 ) min(^{-1} )</td>
<td>0.71</td>
<td>0.24</td>
<td>0.56</td>
<td>0.21</td>
</tr>
<tr>
<td>( B_{max} ) nmol·L(^{-1} )</td>
<td>0.0</td>
<td>0.4</td>
<td>0.0</td>
<td>0.1</td>
</tr>
<tr>
<td>( V_{ND}^* ) ml·cm(^{-3} )</td>
<td>0.8</td>
<td>2.7</td>
<td>0.9</td>
<td>2.5</td>
</tr>
<tr>
<td>Cbm (1CM) ( K_1 ) ml·cm(^{-3})·min(^{-1} )</td>
<td>0.57</td>
<td>0.64</td>
<td>0.51</td>
<td>0.51</td>
</tr>
<tr>
<td>( k_2 ) min(^{-1} )</td>
<td>0.71</td>
<td>0.22</td>
<td>0.54</td>
<td>0.19</td>
</tr>
<tr>
<td>( V_T^{**} ) ml·cm(^{-3} )</td>
<td>0.8</td>
<td>2.9</td>
<td>0.9</td>
<td>2.7</td>
</tr>
</tbody>
</table>

Values in **bold** indicate values the most appropriate model, based on Akaike Information Criterion

\( ^* K_{Dapp} = k_{off}f_{ND}K_{on} \). This is a calculated value based on \( f_{ND}K_{on} \) and \( k_{off} \) and was not a parameter included in the fit to the data

**Values reported for M2 FLB, are averages of estimates in a range; \( k_{on} = 0.1-0.2 \), \( k_{off} = 0.010-0.025 \) and \( K_{Dapp}^* = 0.1-0.2 \)

\( ^* V_{ND} = V_T = K_1/k_2 \) for 1CM. This is a calculated value based on \( K_1 \) and \( k_2 \) and was not a parameter included in the fit to the data

\( ^{**} V_T = K_1/k_2 \) for 2CM. This is a calculated value based on \( K_1 \) and \( k_2 \) and was not a parameter included in the fit to the data
Figure 3-6: Time-activity curves and model estimations.

Free and fixed parameters of $p$ are given on the left.

(A) Summary of model estimation and measured data for the thalamus, substantia nigra, prefrontal cortex, and temporal cortex (in M1).

(B) Summary of cerebellar data fits with both one- and two-compartment models. 1CM is represented by a dotted line and 2CM is shown with a solid line.

Measured data has been re-binned from 30 second frame duration into longer frame durations for illustrative purposes only.
3.4 Discussion

The motivation for these experiments was to perform a direct comparison of the kinetics of two commonly used PET radioligands for assaying extrastriatal D₂/D₃ binding. Although a single bolus injection experimental design is the most feasible for studying changes in receptor-ligand binding (via DVR or BPND) in humans, such a design cannot separate the individual transport and binding processes of the radiotracer. We have chosen to implement this experimentally complex protocol to directly compare and characterize the in vivo rate constants of ¹¹C-FLB457 and ¹⁸F-fallypride to guide experimental design for future studies and to evaluate the strengths and weaknesses of each radiotracer for extrastriatal D₂/D₃ assessment.

3.4.1 Optimization of experimental design

MI experiments must be properly designed for injection timing and proportion of radiolabeled and unlabeled compound to yield estimated parameters that are uniquely identified. However, multiple-injection optimization procedures present a challenge because the procedures used require a priori information about the transport and binding characteristics of a tracer when the goal of the experiments is to determine these very parameters that you need information about.

Based on our previous experience with ¹⁸F-fallypride (Christian et al 2004), it was possible to achieve excellent identification of all the parameters with ¹⁸F-fallypride in both animals. The results of the first ¹⁸F-fallypride study led to the elimination of the third injection based upon a robust design optimization strategy described by Salinas et al. (Salinas et al 2007). However, finding a protocol giving good parameter estimation for ¹¹C-FLB457 was not so straightforward. We acquired two scans with poor
identifiability of \( f_{NDk_{on}} \) and \( B_{\text{max}} \) before finding a protocol that was able to sufficiently identify these parameters of binding.

### 3.4.2 Plasma analysis

Measurements show that \(^{11}\text{C}-\text{FLB457} \) is removed from the blood much more quickly than \(^{18}\text{F}-\text{fallypride} \). Rapid metabolism and clearance of a radioligand from the plasma can be advantageous because it permits a shorter scanning duration to achieve a stable measure of receptor binding. However, the accuracy of the measured arterial concentration is greatly diminished due to low counting statistics due to the \(^{11}\text{C} \) radiolabel.

### 3.4.3 Regional binding and transport

The analysis of the acquired data required multiple-step procedures that applied constraints on parameters such as \( k_{\text{off}} \) and \( B_{\text{max}} \) in order to achieve identifiability of parameters related to binding. Examination of the results reveal that the main differences between tracers lie in their affinity for \( \text{D}_2/\text{D}_3 \) receptors and the rate in which they clear out of tissue from the free space.

Using the averages of both studies, we report an apparent \textit{(in vivo)} \( K_D \) of 0.39 nM for \(^{18}\text{F}-\text{fallypride} \) and 0.13 nM for \(^{11}\text{C}-\text{FLB457} \). This three-fold difference can be attributed to the combination of faster binding and slower dissociation of \(^{11}\text{C}-\text{FLB457} \) as compared to \(^{18}\text{F}-\text{fallypride} \). This difference is relatively consistent with a 2-fold difference in humans (Narendran \textit{et al} 2009) and \textit{in vitro} measures (\( K_D : 0.03 \) nM vs. 0.018 nM, respectively) (Halldin \textit{et al} 1995; Mukherjee \textit{et al} 1995). For fallypride, the measured
apparent dissociation constant is in close agreement with 0.38 nM from previous work in the rhesus monkey (Christian et al 2004) and 0.20 nM in the baboon (Slifstein et al 2004a). For FLB457, literature values have been reported as 0.21 nM in the baboon (Delforge et al 1999) and 0.35 nM (Olsson et al 2004) and 0.9 in humans (Suhara et al 1999).

The delivery of the parent compound from the plasma to the free space of the tissue ($K_1$) was similar for both radiotracers, yielding a mean of $K_1 = 0.48 \pm 0.15$ mL/cm$^3$/min for fallypride and $K_1 = 0.54 \pm 0.10$ mL/cm$^3$/min for FLB457 when averaged across all brain regions. However, there was a large difference in the tissue to plasma efflux constant, $k_2$, with values of $0.54 \pm 0.10$ mL/min for fallypride and $0.18 \pm 0.05$ mL/min for FLB457. Based on these values, it is seen that the nondisplaceable distribution volume ($V_{ND} = K_1/k_2$) is more than 3-fold larger for FLB457 than fallypride; 3.0 compared to 0.89, respectively. This measure of $V_{ND}$ for fallypride is consistent with our previous findings in the rhesus monkey using the MI approach (Christian et al 2004). For $^{11}$C-FLB457, previous MI experiments reported $V_{ND}$ of 0.35 in the baboon (Delforge et al 1999). Recent studies in humans found a $V_{ND}$ of 2.9 for FLB457 (Asselin et al 2007a), which is in closer agreement with the value reported here. This large discrepancy in $V_{ND}$ for FLB457 may be species or methodology dependent; however, it does emphasize the importance of performing radioligand comparisons on the same subjects.

The significant difference in $V_{ND}$ between FLB457 and fallypride cannot be explained by the lipophilicity of the compounds. Generally, higher lipophilicity produces a reduction in the free compound in both the plasma ($f_p$) and brain tissue ($f_{ND}$). Based on literature values using HPLC measurement, fallypride has a log $k_w = 2.43$ (Mukherjee et al 1995) and FLB457 has a log $k_w = 1.89$ (Schmidt et al 1994). In silico measurements of lipophilicity support the higher lipophilicity of fallypride, with an average log P value of
3.3 for fallypride and 2.9 for FLB457, as calculated using ALOGPs (Tetko and Yu 2005). Lipophilicity can be used as an indicator of a compounds ability to cross the blood brain barrier (and yield a higher $V_{ND}$); however, other molecular characteristics must be considered, including molecular size, shape, and ionization potential (Waterhouse 2003).

### 3.4.4 Effect of $k_2$ differences

One of the primary differences in the *in vivo* behavior of FLB457 and fallypride is the tissue to plasma efflux constant ($k_2$). For FLB457, the approximately 3-fold reduction in $k_2$ results in a significantly higher signal in the nondisplaceable (ND) compartment, frequently measured in the cerebellum. Such an increase in this component of the PET signal is advantageous for radiotracers that require an extended PET scanning duration to achieve $BP_{ND}$ stability by improving the statistics in the PET signal. Based on the study of M1 in this work, it was found that at 80 minutes post-injection, the decay corrected $^{11}$C-FLB457 study yielded a cerebellar concentration that was 70% greater than the $^{18}$F-fallypride study, despite having a plasma concentration that was 85% less than $^{18}$F-fallypride. This increased signal in the nondisplaceable compartment also improves visualization of the cortical regions, which can aide in the definition of regions of interest or in spatial coregistration or normalization. However, because the SNR is dependent on measured counts, this advantage of increased $V_{ND}$ is lost for $^{11}$C-FLB457 compared to $^{18}$F-fallypride, due to its shorter half-life.

The $k_2$ parameter also plays a role in the sensitivity of a radioligand for measuring changes in endogenous dopamine competition. Through the use of computer simulations, Morris and colleagues performed a detailed comparison of prospective radioligands available for measuring DA transmission in the brain (Morris and Yoder 2006). In their survey of these radiotracers, it was found that fallypride was almost 3-
fold more sensitive than FLB457 to dopamine displacement. In the case of dopamine displacement, the radioligand is administered prior to a manipulation (i.e. administration of amphetamine) which induces the release of endogenous dopamine, and in turn displaces the radiotracer from the specifically bound receptors. For displacement experimental designs, the PET detection sensitivity of a radioligand will be enhanced by rapid dissociation ($k_{\text{off}}$) and tissue clearance ($k_2$), thus enhancing the contrast in the PET signal before and after dopamine release. For dopamine displacement experiments the faster kinetics of fallypride will improve detection sensitivity, but only in regions where sufficiently high PET signal is present. A recent study by Narendran et al. directly compared $^{11}$C-fallypride and $^{11}$C-FLB457 measured changes in $B_{\text{ND}}$ before and after endogenous dopamine competition in humans using an amphetamine challenge (Narendran et al 2009). Their results found that $^{11}$C-FLB457 demonstrated 30% – 70% higher sensitivity (via $\Delta B_{\text{ND}}$) to dopamine competition than $^{11}$C-fallypride in the cortical regions of the brain. In contrast to the ‘displacement’-type study, this ‘blocking’-type study is designed to have the competing dopamine present prior to the administration of the radioligand. This difference in study design favors $^{11}$C-FLB457 for the blocking type study, because this design is less sensitive to the combination of slow binding dissociation, increased PET signal in both the specifically bound and nondisplaceable states, and the higher proportion in the specifically bound state relative to the nondisplaceable state.

### 3.4.5 Cerebellar kinetics

The cerebellar kinetics are of great importance for PET D$_2$/D$_3$ studies because of their frequent use as a reference region in $B_{\text{ND}}$ determination. In this work, it was found that significant specific binding could be measured in the cerebellum for $^{11}$C-FLB457 but not for $^{18}$F-fallypride.
The presence of specific D₂/D₃ binding in cerebellum has been frequently reported in the literature for 

\(^{11}\text{C-FLB457}\) (Asselin \textit{et al.} 2007b; Delforge \textit{et al.} 1999; Olsson \textit{et al.} 2004), leaving one to conclude that similar issues with specific cerebellar binding would be present with \(^{18}\text{F-fallypride}\). The inability of fallypride to detect specific cerebellar binding in this work can be explained, in part, by its lower \textit{in vivo} affinity compared to FLB457. A ~3x higher apparent Kᵩ of fallypride will result in a 3x lower bound-to-ND fraction at equilibrium. However, the insensitivity to cerebellar binding is also attributed to several other characteristics of fallypride, including the reduced radiotracer clearance from the blood, faster k₂ and slower rate of receptor-ligand association (f_{ND}k_{on}) as compared to FLB457.

To investigate the sensitivity of both tracers to a small level of cerebellar binding, we compared simulations of the cerebellum-to-plasma ratios for both radiotracers assuming a receptor density of 0.1 nM, as shown in Figure 3-7. In the absence of specific binding, this ratio should plateau at V_{ND}. In the presence of specific binding, the ratio will plateau at V_T ( = V_{ND} + V_S) (as simulated in Figure 3-7). The distinctive upward bend in the FLB457 curve is due to the fast f_{ND}k_{on} combined with the rapidly declining plasma concentration. It can be seen that this shape in the curve is enhanced by the slow k₂ of FLB457. Thus the slow clearance of FLB457 from the tissue acts as a leaky integrator of the radiotracer, enhancing the opportunity for the ligand to specifically bind to the receptor sites. This shape is not seen with fallypride because of the combination of slower plasma clearance of the parent ligand and a slower rate of specific binding.
Figure 3-7: Simulated cbm/plasma curves

Cerebellum to parent plasma ratios assuming a cerebellar D$_2$/D$_3$ receptor concentration of 0.1 nM. (A) Simulated cerebellum/plasma curves using FLB457 measured plasma data. (B) Curves generated using measured fallypride plasma concentration. Both fallypride-like kinetics and FLB457-like kinetics are given using each plasma input function. Variations in $k_{on}$ from the tracer’s true $k_{on}$ value (±50%) are shown by dotted and dashed lines. Thick solid lines indicate the kinetics parameters of FLB457 and FLB457 blood curve (A) and fallypride kinetic parameters and fallypride blood curve (B).
3.5 Conclusions

There is a high degree of similarity in the visual appearance of the PET images of $^{18}$F-fallypride and $^{11}$C-FLB457, with both radiotracers yielding similar target to background ratios throughout the brain regions with differing D$_2$/D$_3$ receptor densities. However, assessing the performance of a radiotracer based simply on target to background ratios can lead to a misguided understanding of its ability to provide an accurate measure of receptor-ligand binding (Eckelman et al 2009). Compared to fallypride, FLB457 exhibits faster ligand-receptor binding and slower dissociation from the receptor, translating into an equilibrium dissociation constant that is approximately 3 times lower. Fallypride clears from the nondisplaceable space faster and remains in the plasma for longer than FLB457. The higher affinity and nondisplaceable component of FLB457 will provide higher tracer uptake in the cortical and cerebellar regions, however, this will be offset by the lower counting statistics of the $^{11}$C radiolabel. The presence of cerebellar binding may preclude the use of reference region methods for cortical D$_2$/D$_3$ assay.
3.6 References


Eckelman WC, Kilbourn MR, Mathis CA (2009) Specific to nonspecific binding in radiopharmaceutical studies: it's not so simple as it seems! *Nuclear Medicine and Biology* 36:235-7


ALOGPS (HTTP://WWW.VCCLAB.ORG) is a free on-line program to predict lipophilicity and aqueous solubility of chemical compounds. Proceedings of the 229th National Meeting of the American-Chemical-Society, Mar 13-17 2005; San Diego, CA

Chapter 4

Pharmacological Manipulations of $D_2/D_3$ Binding

4.1 Introduction

A common goal of PET neuroreceptor imaging is to detect changes or differences in the underlying physiology between groups of subjects or within subjects, as measured through a change in radioligand binding. In this chapter, we pharmacologically induce changes in PET signal and further our comparison of FLB457 and fallypride by examining their sensitivities to these changes.

Blocking of $D_2/D_3$ receptors via an antipsychotic agent is known to alter radioligand binding. One use of a blocking type experiment is to determine the percent occupancy for a receptor site by drug through comparing measured nondisplaceable binding potential ($BP_{ND}$) before and after drug treatment. This method can provide an alternative to large scale clinical trials, helping to determine appropriate doses for antipsychotic drug treatments, by comparing a measured binding potential before and after drug treatment. The relative percent change in $BP_{ND}$ is an estimate of the percentage of receptors occupied by the drug, assuming a constant $K_D$. Percent occupancy is important because there is an window of between 65% and 80% receptor occupancy by antipsychotic (Kapur and Mamo 2003; Tauscher and Kapur 2001) where treatment is most effective. If the dose is too low and occupancy falls below this window, a treatment is often not effective, and if a dose gives occupancy greater than 80%, patients often experience extrapyramidal side effects.
While treatment with antipsychotics leads to a decrease in radioligand binding, pharmacological depletion of endogenous dopamine will lead to an increase in radioligand binding. Using either α-methyl-para-tyrosine (AMPT) or reserpine, dopamine depletion, as measured by PET, has been reported in normal populations of rats (Seneca et al 2008), baboons (Delforge et al 2001), and humans (Riccardi et al 2008). PET measurements after dopamine depletion is of particular interest because many psychiatric disorders are thought to arise from non-normal levels of synaptic dopamine. Comparing a group of normal controls to a group of schizophrenic patients, Abi-Dargham showed that an increase in SPECT signal was larger in schizophrenia patients than controls after depletion with AMPT, providing evidence that schizophrenia is associated with an increased level of synaptic dopamine (Abi-Dargham et al 2000).

To investigate the differences between fallypride and FLB457 binding following pharmacological manipulations, we performed experiments to both decrease and increase radioligand binding. For inducing a decrease in PET signal, we used haloperidol to block D₂/D₃ receptors. It is a commonly used neuroleptic that is not thought to have differential effects across different regions. Alternatively, non-blocking manipulations such as d-amphetamine or methylphenidate would also lead to a decrease in PET signal due to an increase in synaptic dopamine. These methods, however, d-amphetamine does not give a sustained change in dopamine (Moghaddam et al 1993) and methylphenidate has been shown to only induce a very small change in radioligand binding (Montgomery et al 2006).

Reserpine and α-methyl-para-tyrosine (AMPT) are two commonly used pharmacological agents used in animal models that induce dopamine depletion and an associated increase in radioligand binding. Reserpine acts by blocking the vesicular monoamine transporter, which leads to less dopamine contained in presynaptic vesicles, leading to reduced levels of synaptic dopamine (Quinn et al 1959). AMPT acts by
inhibiting tyrosine hydroxylase, the first of the two enzymes involved in the synthesis of dopamine from the amino acid tyrosine (SjoerdsmA et al 1965). The decrease in dopamine synthesis then leads to lower concentrations of synaptic dopamine, which has been quantified by in vivo microdialysis (Watanabe et al 1997). In this work, we chose to use AMPT as opposed to reserpine to avoid the long term effects (> 20 days) of reserpine (Ginovart et al 1997).

In this chapter, we also introduce 11C-fallypride (Mukherjee et al 2004). This 11C-labelled version of fallypride is presumed to have identical imaging properties to 18F-fallypride, except for its half-life. The short half-life (20.4 minutes) is an advantage for performing multiple PET studies in the same subject in a single day, something that cannot be performed with 18F-fallypride. This is an advantage in studies where a research subject serves as its own control, eliminating the need to obtain PET scans on separate days. The short half-life, however, can be a disadvantage in that it limits the useful duration of a scan. This is particularly important in regions of low binding, such as the cerebellum, where low count rates lead to a poor signal to noise ratio.

The goals of the experiments addressed in this chapter are three-fold. First, we look to quantify receptor occupancy with both 11C-FLB457 and 11C-fallypride due to a small dose of haloperidol. Second, we aim to investigate the sensitivity of both tracers to dopamine depletion via administration of AMPT. Finally, we investigate the minimal scan length needed for a stable estimate of cortical binding potential using both radioligands.
4.2 Experimental procedures

Experiments were conducted on three rhesus monkeys (M1: 5 years, 5 kg; M2: 5 years, 5 kg; M3: 5 years, 7.5 kg). M1 and M2 had six PET scans each, conducted in three separate imaging sessions, while M3 had four PET scans over two imaging sessions. A summary of scanning sessions is given below in Table 4-1.

<table>
<thead>
<tr>
<th></th>
<th>Scan Session #1</th>
<th>Scan Session #2</th>
<th>Scan Session #3</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>FLB457 → hal → FLB457</td>
<td>fallypride → hal → fallypride</td>
<td>AMPT → fallypride → FLB457</td>
</tr>
<tr>
<td>M2</td>
<td>FLB457 → hal → FLB457</td>
<td>fallypride → hal → fallypride</td>
<td>AMPT → FLB457 → fallypride</td>
</tr>
<tr>
<td>M3</td>
<td>FLB457 → hal → FLB457</td>
<td>fallypride → hal → fallypride</td>
<td>n/a</td>
</tr>
</tbody>
</table>

(FLB457 = $^{11}$C-FLB457; fallypride = $^{11}$C-fallypride, hal = haloperidol)

4.2.1 Radiochemistry

Radiochemical synthesis followed procedures given in Chapter 2. Separate syntheses were performed for the first and second emission scans. At the time of injection, the specific activity of $^{11}$C-fallypride was $750\pm260$ mCi/umol, and $1200\pm700$ mCi/umol for $^{11}$C-FLB457.

4.2.2 PET scans and data acquisition

Prior to PET scanning, the monkey was initially anesthetized with 10 mg/kg ketamine (i.m.) and intubated. Following intubation, the monkey’s anesthesia was continued with isoflurane at 0.75-1.5% for the entire scanning session. To help maintain normal body temperature, a BairHugger® blanket was
placed on top of the monkey. Vitals including heart rate, breathing rate, body temperature, and SpO₂ were monitored for the entire study period. After intubation and start of isoflurane administration, the animal was positioned in a stereotactic head-holder, and a transmission scan was acquired for 518 seconds using a Co-57 point source.

All imaging sessions consisted of two 90 minute emission scans, initiated by the bolus injection of radioligand. The second scan was initiated approximately 30 minutes after completion of the first emission scan. For each monkey, the imaging sessions were separated by at least two weeks.

### 4.2.3 Haloperidol and AMPT administration

Haloperidol was obtained from the UW hospital pharmacy (50mg/10mL) and diluted to a concentration of 100 µg/mL with 0.9% bacteriostatic NaCl. For the haloperidol imaging sessions (#1 & #2), 20µg/kg haloperidol was administered via a subcutaneous injection in the posterior portion of the monkey’s lower leg, 20 minutes prior to the beginning of the second scan.

α-methyl-DL-tyrosine methyl-ester hydrochloride (AMPT, CAS# 7361-31-1; Sigma-Aldrich, St. Louis, MO) was dissolved in 0.9% bacteriostatic NaCl at a concentration of 67 mg/mL. pH was adjusted to pH = 6.0 by addition of ~3 drops 5 N NaOH per 10 mL saline, resulting in a transparent solution with a slight yellow tint. While not used in this experiment, it is worth noting that we also worked with the non-salt, amino-acid form of AMPT (α-methyl-DL-tyrosine, CAS# 658-48-0). The amino acid form is not readily soluble in water, requiring a pH below 3 or above 12 for it to go into solution. At pH levels between 3 and 12, the AMPT precipitates, creating a white, cloudy solution, rendering it unsuitable for injection. In the mornings before both AMPT imaging sessions (#3), the monkey was removed from its home cage and
transferred to a squeeze cage where AMPT was administered I.V. through a 24g catheter in the saphenous
vein of the awake monkey. For M1, AMPT was administered 5 hours and 15 minutes prior to the first
emission scan. For M2, the delay was 4 hours and 45 minutes.

4.2.4 Image processing and data analysis

Histogramming of emission data produced dynamic sinograms, making corrections for deadtime and
randoms, each with 27 frames (frames x minutes per frame; 4x0.5, 3x1, 3x2, 3x3, 14x5 ). The emission
sinograms were reconstructed using filtered backprojection applying a 0.5 cm$^{-1}$ ramp filter, zoom of 1.5x,
resulting in images with a 128x128x63 voxel matrix size with voxel size of 1.26mm x1.26mm x1.21mm.
To create images with quantitative units of nCi/cc, corrections were made for attenuation, decay, scanner
normalization, and scatter. Using FSL flirt (Smith et al 2004), AMPT scans were transformed to be in the
same orientation as the corresponding baseline and haloperidol scans.

For the generation of time-activity curves, regions of interest (ROIs) were drawn in on a summed data of
the first 3 minutes of the baseline scan. Using methods similar to those described in the previous chapter,
ROIs were drawn over the cerebellum, prefrontal cortex, thalamus, and amygdala by placing circular
ROIs within the boundaries of each region. As ROIs spanned multiple slices, ROI placement was
determined within each slice. The set of ROIs for each study was applied to each of three dynamic image
sets for each tracer for each monkey, generating time-activity-curves (TACs) to be used for kinetic
analysis. Cerebellar time-activity curves were fit to dual-exponential decay functions, with the fit data
replacing the measured data points after 3 minutes. Time-activity curve data was re-processed to give
frame durations up to 15 minutes (number of frames x minutes per frame;  5x1, 3x2, 3x3, 4x5, 2x10,
2x15 ) to reduce the level of noise in the TACs.
**Logan Data Analysis**

Data analysis was performed using the Logan graphical reference region model (Logan 2000), using the cerebellum as the reference region. This method applies transformations to the measured time activity curves, linearizing the data for late time periods according to the relationship below.

\[
\int_0^T C(t) dt \quad \frac{C(T)}{C(T)} = DVR \left[ \int_0^T C'(t) dt + \frac{C'(T)}{k_{REF}} \right] + \text{int}'
\]

C(t) is the concentration (nCi/cc) in receptor-rich tissue at time t, and C'(t) is the concentration in the reference region. All data points after T* = 30 min were fit with simple least squares linear regression, resulting a values for the slope and Y intercept, with the slope representing an estimate of the distribution volume ratio (DVR). DVR is equivalent to the non-displaceable binding potential plus one (DVR = BP_{ND} + 1).

**Haloperidol Occupancy, Change in BP_{ND} with AMPT**

In both haloperidol and AMPT studies we report a percent change in BP_{ND} from baseline, as given below.

\[
\%Occ = \Delta B_{P_{ND}}^{drug} \times 100\% = \frac{B_{P_{ND}}^{drug} - B_{P_{ND}}^{baseline}}{B_{P_{ND}}^{baseline}} \times 100\%
\]

With the haloperidol studies, |\%Occ| can be interpreted as the percent of D_{2}/D_{3} receptors occupied by haloperidol.
4.3 Results

The results from the haloperidol studies are given on in Figure 4-1, Figure 4-2, and Figure 4-3. The table accompanying each plot gives values for $BP_{ND}$ and the percent change from baseline in the two drug conditions. Each figure gives TACs for each of the three regions; PFC, thalamus, and amygdala. To account for differences in injected activity and blood flow differences between scans, all time-activity curves shown are scaled to the average integrated cerebellar radioactivity. An interval of 7 minutes was chosen for the integration period on the cerebellum as it represented a period where the cerebellum signal was dominated by signal from unbound radiotracer.
Figure 4-1: Prefrontal Cortex TACs

The y-axes represent normalized radioactive concentration. The x-axes represent time from injection. Solid lines represent the cerebellum curves.
Figure 4-2: Thalamus TACs

The y-axes represent normalized radioactive concentration. The x-axes represent time from injection. Solid lines represent the cerebellum curves: Blue-haloperidol, black-baseline, red-AMPT.
**Figure 4-3:** Amygdala TACs

The y-axes represent normalized radioactive concentration. The x-axes represent time from injection. Solid lines represent the cerebellum curves: Blue-haloperidol, black-baseline, red-AMPT.
4.4 Discussion

Drug Dosing and Delivery

The dose of 20µg/kg haloperidol was chosen based on a study performed by Mukherjee et al., which reported 15% occupancy at 10µg/kg haloperidol (i.p) and 80% occupancy at 100µg/kg in rat striatum (Mukherjee et al. 2001). They also reported that 50µg/kg haloperidol results in occupancies of 83-91% across regions of high to low binding in the rhesus monkey. With our dose being 60% lower than 50µg/kg, we would have expected a lesser degree of occupancy than we measured.

Selection of dosing and delivery of AMPT was more complicated. Of particular concern is the toxicity of AMPT, with a reported LD$_{50}$ in mice of 400 mg/kg (Sofia and Knobloch 1974) and no toxicity data in non-human primates. The dose of 100mg/kg was chosen as a conservatively based on a study of 16 dogs where 114 mg/kg was given intravenously with no side effects (Luthra et al. 1981). Other considerations included the route of administration and the timing of dose relative to scan start. Intravenous delivery was chosen as the best route of administration due to concerns with other methods. Due to the relatively low solubility of AMTP methyl-ester HCl (<100mg/mL), the volume of injected solution (~10 mL) would be too large for safe intramuscular, intraperitoneal, or subcutaneous injections. Despite a large number of studies reporting a significant amount of dopamine depletion following an oral regimen AMPT (Abi-Dargham et al. 2000; Boot et al. 2008; Riccardi et al. 2008; Voruganti et al. 2001), oral delivery was ruled out due to the need to food deprive the monkey for 12 hours prior to each scanning session and because we were limited to a single acute dose due to protocol limitations. The delivery timing of five hours prior to the first emission scan was chosen based on two reports in the literature, with the first showing that concentration of dopamine in the rat striatum is still decreasing at 4 hours following systemic
administration of AMPT (Watanabe et al 2005) and the second reporting that changes in dopamine were still present 18 hours following i.v. AMPT administration (Leitner and Roumy 1986).

**Occupancy results**

Both $^{11}$C-fallypride and $^{11}$C-FLB457 gave very similar measures of haloperidol occupancy for each individual monkey, with the average difference in occupancy measures across tracers being $7.2\pm8.5\%$. The relationship between the measures of occupancy across tracers is plotted below in Figure 4-4. The plot shows the close agreement between measures of occupancy across tracers, with the PFC showing the best agreement.
Figure 4-4: Comparison of measured haloperidol occupancies with $^{11}$C-fallypride and $^{11}$C-FLB457. The dotted line indicates the line of identity.

Occupancy measures were not, however consistent across animals in the PFC and amygdala, suggesting that there is a large degree of biological variation across animals in these regions (PFC, 24-67%; amygdala, 60-100%). The thalamus demonstrated consistent measures of occupancy across animals and tracers (78-89%). This level of occupancy agrees with the work done with $^{11}$C-FLB457 in a single rhesus monkey, where 10 µg/kg and 30 µg/kg (i.v.) have associated cortical occupancies of 65% and 76%, respectively (Takano et al 2004). We see that occupancy is, on average, lower in the prefrontal cortex than in other regions. The differential occupancy measured across regions follows with that reported by
Talvik et al. (Talvik et al 2001), but not that of Xiberas et al. (Xiberas et al 1999), who report similar occupancy across regions after oral haloperidol administration.

In both fallypride AMPT studies (M1 and M2) and in the M2 FLB study, the TACs in all regions suggest that there is more specific binding after AMPT, as would be expected after depletion of endogenous dopamine. However, the results of the Logan analysis do not support the theory AMPT leads to more available receptors, for the linearization gives DVR values of less than baseline in most regions. As the dose was relatively low compared to other dosage studies and that five hours passed between AMPT administration and the first scanning session, it is possible that the drug administration method did not provide sufficient depletion of endogenous dopamine to be detectable using our methods. The lack of measured effect could be a consequence of a bias inherent in reference region methods that use a reference region containing specific binding sites, as discussed below.

*Effects of Cerebellar Binding*

With both tracers and in all three monkeys, we see a decrease in signal after haloperidol administration, suggesting that there is indeed cerebellar binding to an extent with both tracers. This observation contrasts what we observed in the previous chapter using the multiple injection technique, where there was no evidence of cerebellar binding with $^{18}$F-fallypride. The signal attributed to specific binding in the cerebellum is likely to be much greater for $^{11}$C-FLB457 based on the greater separation between baseline and haloperidol curves, but quantification of cerebellar binding potential is only possible with using an arterial input function.
Reference region methods are commonly used in quantitative neuroreceptor imaging due to their relative simplicity relative to methods requiring measurement of an arterial input function. In D₂/D₃ neuroreceptor imaging, the cerebellum is often used as the reference region, due to its very low concentration of receptors. However, there is particular interest as to whether or not the use of the cerebellum as a reference region is valid when using high affinity radioligands. Quantification of cerebellar binding has been addressed in a variety of papers, each drawing conclusions that the use of reference regions with high-affinity radioligands will likely lead to an underestimation of nondisplaceable binding potential (Asselin et al 2007; Christian et al 2004; Delforge et al 1999; Pinborg et al 2007).

To quantify the expected error in a measurement of nondisplaceable binding potential using reference region methods, we look at it algebraically, in a similar method to that given previously (Asselin et al 2007). When there is specific binding in the reference region, nondisplaceable binding potential is underestimated. The relationship between the measured nondisplaceable binding potential (BPₐ₅ = DVR-1) and true binding potential (k₃/k₄) is given below.

\[
\frac{BP_{ND}}{BP_{true}} = \left[ \frac{BP_{measured}}{BP_{true}} \right] = \left[ \frac{(DVR-1)}{k_3/k_4} \right]
\]

Substituting in the definitions of both distribution volume ratio and of k₃, then performing algebraic manipulations, we see the inherent error in using this method as a function of the receptor density in tissue, the receptor density in the reference region, and the binding potentials in tissue and the reference region. When the data fits the assumptions to the model (B_{max,ref} = 0) there is no error, as expected.
\[
DVR = \frac{\left(1 + \frac{k_{\text{tissue}}}{k_4}\right)}{\left(1 + \frac{k_{\text{ref}}}{k_4}\right)}
\]

\[
k_3 = k_{\text{on}}B_{\text{max}}
\]

\[
\frac{\text{BP}_{\text{ND}}}{\text{BP}_{\text{true}}} = \left[1 - \frac{B_{\text{max,ref}}\left(1 + \text{BP}_{\text{ND,ref}}\right)}{B_{\text{max,tissue}}\left(1 + \text{BP}_{\text{ND,tissue}}\right)}\right]
\]

The above equation also shows that for the same level of receptor density as measured using different ligands, the underestimation of \( \text{BP}_{\text{ND}} \) is amplified by a greater degree of binding, making the underestimation greater for high affinity radioligands. This idea is illustrated in Figure 4-5, below, where we show the error in \( \text{BP}_{\text{ND}} \) in regions of \( B_{\text{max}} = 0.5, 1.5 \) and 3.0 across a range of receptor densities in the reference region (0-0.5 nM). The two families of curves represent fallypride and FLB457.
**Figure 4-5:** Theoretical underestimation of $\text{BP}_{\text{ND}}$ using reference methods

Series of plots are theoretical regions of cortex (*, $B_{\text{max}} = 0.5$), thalamus (○, $B_{\text{max}} = 1.5$), and SN (×, $B_{\text{max}} = 3.0$). Fallypride curves are black, FLB457 curves are gray.
Christian et al. take this one step beyond calculating error in $B_{\text{ND}}$ (Christian et al. 2004), but look at the expected error in a measure of occupancy, assuming equal receptor occupancy in both receptor-rich tissue and reference tissue, as given by:

$$Occ_{\text{error}} = Occ_{\text{true}} - Occ_{\text{app}}$$

$$Occ_{\text{error}} = \left[1 - \frac{BP_{\text{drug}}}{BP_{\text{baseline}}}\right] - \left[1 - \frac{BP_{\text{drug}}}{BP_{\text{baseline}}} \left(\frac{BP_{\text{ref}}}{BP_{\text{drug}} + 1}\right)\right] \times 100\%$$

This equation shows that the apparent occupancy will be less than the true occupancy when there is cerebellar binding, and the degree of this error is a function of the true occupancy and $BP_{\text{ref}}$. Considering this, we must assume that the calculated occupancy measures due to haloperidol reported above are underestimations of the true occupancy.

We also notice that despite the reported higher affinity of $^{11}$C-FLB457, we do not see higher binding potentials than for $^{11}$C-fallypride for the same regions. This is perhaps because the estimation of $B_{\text{ND}}$ is greater with $^{11}$C-FLB457 than with $^{11}$C-fallypride due to a larger bound signal in the cerebellum. For example, in a region with a concentration of receptors of 0.5 nM, we would expect $^{11}$C-fallypride ($K_{\text{Dapp}} = 0.35$) to have a nondisplaceable binding potential of 1.4 and $^{11}$C-FLB457 ($K_{\text{Dapp}} = 0.35$) to have a nondisplaceable binding potential of 3.3. However, if you account or a cerebellar binding at 0.2 nM, $^{11}$C-FLB457 is underestimated by 75% while $^{11}$C-fallypride is underestimated by only 62%, bringing their measured binding potentials into closer agreement at 0.54 and 0.83, respectively.
**Scan Duration**

To determine the minimal scan duration needed to obtain a stable estimate of cortical nondisplaceable binding potential ($BP_{ND} = DVR - 1$), we used our 90 minute data sets and remove up to 50 minutes of data from the end of the scan, in 5-minute intervals. Binding potential is then calculated for each subset of the full data set. Results of $BP_{ND}$ calculations are presented as the percentage of the binding potential as calculated using 90 minutes of data, as the data is shown below in Figure 4-6.

$$\%BP = \left(1 - \frac{BP(t = 90) - BP(t)}{BP(t = 90)}\right) \times 100\%$$

The results from these experiments show that for both $^{11}$C-FLB457 and $^{11}$C-fallypride, estimation of cortical $BP_{ND}$ remained stable within $\pm 5\%$ of the $BP_{ND}$ found using 90 minutes of data, with only using 75 to 80 minutes of data, which serves as a lower-limit for an acceptable scan duration.
**Figure 4-6**: Scan duration analysis.

The y-axis represents the measure of $\text{BP}_{\text{ND}}$ relative to the measure of $\text{BP}_{\text{ND}}$ using 90 minutes of data. Blue points represent $^{11}\text{C}-\text{FLB457}$ scans. Red points represent $^{11}\text{C}$-fallypride scans. The data point shape designates dataset used ($\triangle = \text{M1}$, $\ast = \text{M2}$, $\circ = \text{M3}$). Black lines represent 100%±5%.

**Cerebellar Binding**

In the previous chapter, we discuss the how plasma clearance rate, tissue clearance rate, and tracer half-life affect the signal-to-noise ratio of $^{18}\text{F}$-fallypride and $^{11}\text{C}$-FLB457 in the cerebellum, finding that the non-decay corrected radioactivity concentration of $^{11}\text{C}$-FLB457 is 70% lower than that of $^{18}\text{F}$-fallypride at
80 minutes post-injection. When we compare $^{11}$C-fallypride to $^{11}$C-FLB457, however, we find that $^{11}$C-FLB457 has a count rate of 5 to 10 times higher than $^{11}$C-fallypride at 80 minutes post injection, as shown in Figure 4-7. Considering the effects of radioactive decay alone, $^{18}$F-fallypride should have a count rate 9 times that of $^{11}$C-fallypride at 80 minutes for the same injected activity. Figure 4-7 plots non-decay-corrected time activity curves in the cerebellum for both $^{11}$C labeled tracers and $^{18}$F-fallypride.

**Figure 4-7:** Baseline scan cerebellum curves comparing radiotracers

Cerebellum curves averaged across the three baseline scans for both $^{11}$C-fallypride and $^{11}$C-FLB457, scaled to injected activity. The $^{18}$F-fallypride curve is from data collected in the experiments from Chapter 3. Decay correction has been removed from all data sets.

Subplot shows data from 50-90 minutes with zoom applied to y-axis.
4.5 Conclusion

The goal of the above experiments was to characterize the differences between $^{11}$C-fallypride and $^{11}$C-FLB457 with respect to their sensitivity to pharmacologically induced changes in receptor availability. This was performed by using the antipsychotic haloperidol as a $D_2/D_3$ blocking agent, providing between 24% to 100% blocking of receptors. Higher occupancies were seen in the thalamus and amygdala than in the prefrontal cortex. Measures of drug occupancy were in close agreement within each animal across tracers, with an average difference between tracers of 7% ± 9%. A conservative dose of 100 mg/kg AMPT was used to deplete levels of endogenous dopamine, leading to inconclusive results on whether or not this led to a greater availability of receptors available for radioligand binding. This is, in part, due to variations in the degree of binding in the reference region (cerebellum) across different drug treatments, which induces an error in the estimate of DVR, with the error being larger for $^{11}$C-FLB457 than $^{11}$C-fallypride due to the higher affinity of FLB457. An investigation of the scan time needed for accurate measurement of cortical DVR was performed, providing evidence that at least 75 minutes of data should be collected for both $^{11}$C-FLB457 and $^{11}$C-fallypride scans. With these results, we can conclude that while both $^{11}$C-fallypride and $^{11}$C-FLB457 give similar results in response to drug treatment, the larger PET signal in late data frames suggests that $^{11}$C-FLB457 is better suited for back to back, drug-manipulation PET studies.
4.6 References


Chapter 5

Electrophysiological Manipulations of D$_2$/D$_3$ Binding

5.1 Introduction

Previous chapters concentrated on characterizing and comparing fallypride and FLB457 using both multiple-injection techniques and pharmacological manipulations. In this chapter, we measure changes in D$_2$/D$_3$ receptor availability brought about by electrophysiological manipulations. Specifically, we use $^{18}$F-fallypride to image changes of the dopamine system in a non-human primate undergoing chronic electrical stimulation of the bed nucleus of the stria terminalis.

We evaluated the utility of using a high-affinity radioligand for whole-brain neuroreceptor imaging, as applied to during deep brain stimulation (DBS) research. DBS treatment involves surgically implanting electrodes into a deep brain structure such as the subthalamic nucleus, globus pallidus or thalamus followed by chronic electrical stimulation of the target nucleus. This treatment can effectively alleviate symptoms associated with movement disorders such as Parkinson’s disease, essential tremor, and dystonia; often substantially increasing the patient’s quality of life. Beyond its clinical applications, DBS has also been used experimentally in attempts to treat epilepsy, depression, obsessive-compulsive disorder, cluster headache, and obesity (Hamani et al 2008; Lacan et al 2008; Leone et al 2005; Mayberg et al 2005; Perlmutter and Mink 2006; Sani et al 2007). Despite its many uses, the mechanisms of DBS effectiveness remain unclear (McIntyre et al 2004; Montgomery and Gale 2008). With a better understanding of the mechanisms of DBS, treatments could be implemented more effectively and new applications may be discovered. Much of the current research in DBS uses electrical recording on the
cellular level, but a more systems level approach, such as molecular imaging, shows promise as a research tool for understanding the neurochemical changes accompanying DBS treatment.

Functional imaging shows great potential for investigating the mechanism of DBS through measurement of regional blood flow, imaging of regional metabolism or other biochemical processes, and via measurement of neureceptor concentrations. Using $^{15}$O-H$_2$O to measure changes in regional blood flow in essential tremor patients, Perlmutter et al. found that thalamic stimulation increased blood flow in targets downstream of the thalamus (Perlmutter et al. 2002). Also using $^{15}$O-H$_2$O, Haslinger et al. examined patients with DBS of the ventralis intermedius, measuring increases in blood flow at the stimulation site and sensory motor cortex, both correlated with stimulus frequency and stimulus amplitude (Haslinger et al. 2005). Using $^{18}$F-FDG, Fukuda et al. observed metabolic changes correlated with changes in the Unified Parkinson’s Disease Rating Scale scores during pallidal DBS (Fukuda et al. 2001), and Hilker et al. found that DBS of the subthalamic nucleus activates the stimulated target while altering non-motor circuits (Hilker et al. 2004). Furthermore, the experiments by Schlaepfer et al. showed that DBS of the nucleus accumbens alters metabolism in a distributed network of limbic and prefrontal brain regions (Schlaepfer et al. 2007).

To gain further insight into the physiological mechanisms of DBS, beyond regional perfusion and metabolism, neuroligand PET methods offer great potential to examine specific biochemical processes during DBS. The dopaminergic neureceptor system is of particular interest with DBS treatment of movement disorders. To date, neuroligand PET has been limited to only studies of the striatum including three PET studies on patients with DBS of the subthalamic nucleus. All three came to the conclusion that stimulation of the subthalamic nucleus does not significantly alter $^{11}$C-raclopride binding to D$_2$/D$_3$
receptors in the striatum (caudate and putamen) (Abosch et al 2003; Hilker et al 2003; Strafella et al 2003). However, another study reported significant \textsuperscript{11}C-raclopride binding differences between pre- and post-DBS surgery groups, suggesting that DBS of the subthalamic nucleus reduces levadopa-induced fluctuations of synaptic dopamine levels in the striatum (Nimura et al 2005). These studies did not provide information about regions outside of the striatum due to the moderate affinity of \textsuperscript{11}C-raclopride for D\textsubscript{2}/D\textsubscript{3} receptors. As demonstrated in the previous chapters, \textsuperscript{18}F-Fallypride is a high affinity D\textsubscript{2}/D\textsubscript{3} radioligand that provides favorable imaging characteristics for striatal and extrastriatal regions of the brain, making it a useful radioligand for exploring system-wide changes in the dopaminergic network.

In this study, \textsuperscript{18}F-fallypride was used to track changes in D\textsubscript{2}/D\textsubscript{3} receptor binding as a result of DBS of the bed nucleus of the stria terminalis (BNST). Chronic stimulation of the BNST was explored as a mechanism for regulating the feeding habits of a naturally obese rhesus monkey. Previous work has shown that the BNST has projections to a variety of dopaminergic neurons (Fudge and Haber 2001) and that lesions of limbic system components closely related to the BNST have led to hyperphagia and obesity in rats (stria terminals (Rollins et al 2006); posterior dorsal amygdala (King 2006)). A reduction in D\textsubscript{2}/D\textsubscript{3} receptor availability has been reported in obese humans, suggesting a deficiency in the modulating role of dopamine in motivational and reward systems in obese subjects (Wang et al 2001). The BNST is implicated in modulating dopamine transmission in these systems (Norgren et al 2006).

Using \textsuperscript{18}F-fallypride, we conducted the first PET imaging study using a high-affinity radioligand with an application to deep brain stimulation (Vandehey et al 2009). We show that DBS causes an increase in radioligand binding due to chronic stimulation and that after a period of over three weeks, \textsuperscript{18}F-fallypride binding returns to its baseline levels. Second, we demonstrate that while there are regional increases in
binding at both high and low frequency stimulation as determined by ROI analysis, there are also sub-regional differences within the ROI boundaries. Finally, we give evidence that the increases in binding are not immediate, suggesting that they occur over a longer time period than can be detected in a single PET scan. While each of these results, taken individually, may provide an insight into the mechanisms of DBS, when taken together, they demonstrate the utility of quantitative PET neuroimaging for detecting changes as brought about by electrophysiological manipulation.

5.2 Experimental procedures

5.2.1 DBS surgery and stimulation parameters

The experiment involved repeated scans of a male rhesus monkey (macaca mulatta: 6 yrs, 15 kg). Experimental procedures were approved by the UW Institutional Animal Care and Use Committee. Magnetic resonance imaging (MRI) images of the brain were acquired before and after surgery; pre-surgery images were used for surgical planning, post-surgery images for aiding in determination of the stimulated structure. The DBS lead was surgically implanted in the right bed nucleus of the stria terminalis (BNST), corresponding to the coordinates of x = 3.3mm, y = 3.85 mm (posterior to the anterior commissure), z = 0.55mm (above AC-PC plane) of the Paxinos rhesus atlas (Paxinos 1999). Electrode location was later confirmed based upon histological sections following the experiments. After surgery, the animal was allowed to recover for two months before the acquisition of the post-surgery MRI and PET scans. A T1-weighted MRI showing DBS electrode placement is shown in Figure 5-1.

The electrode waveform generator (located in the thorax) was set to deliver electrical pulses to the two most distal contacts of the electrode with a pulse width of 60 microseconds. Frequency was set to either
130 or 50 Hz using a pulse amplitude of 0.5, 1.0 or 2.0V. These stimulation parameters were chosen because they are commonly used clinical stimulation patterns. As stimulation frequency has been shown to influence effectiveness of clinical DBS treatment (Moro et al. 2002; Windels et al. 2003), stimulation using both 130 Hz and 50 Hz was applied to investigate the frequency dependence on BNST stimulation response.

![Figure 5-1: DBS lead placement and scale](image)

The MRI (left) indicates the post-surgical placement of the electrode. Due to susceptibility artifacts the tract appears much larger than the actual electrode. The histology slice (right) illustrates the entry point and the partially removed DBS lead and shows the size of the electrode.

### 5.2.2 Timing of PET scans and stimulation parameter changes

The timing of PET scans relative to stimulation voltage and frequency is shown in Figure 5-2a. The first 130Hz (high frequency) stimulation period consisted of four weeks of constant stimulation at 0.5V followed by four weeks with the system turned off, during which service was performed on the waveform
generator. After the service period, the stimulators were turned on again at 130Hz; four weeks at 1.0V and another four weeks at 2.0V. Subsequently, the stimulator was turned off for four weeks (washout period), followed by twelve weeks of stimulation at 50Hz. This twelve-week low-frequency period was split into three 4-week segments with the voltage set to 0.5, 1.0 and 2.0V, respectively. Following the 50Hz period, the stimulator remained off for a second washout period. All PET scans were acquired within five days of the end of each time period. The final PET scan was acquired 4.5 weeks after 50Hz stimulation ended. The timing of on/off sessions was determined by the neurosurgeon, as part of the underlying DBS experiment on obesity. The dates of PET scanning were chosen as times with the highest chance for detecting a change in radioligand binding.

![Diagram of DBS timeline, striatal DVR measure and monkey weight](image)

**Figure 5-2:** DBS timeline, striatal DVR measure and monkey weight

(a) Timeline showing stimulation duration, strength, and frequency. Scanning times are indicated above the timeline. (b) Weight and striatum $^{18}$F-fallypride DVR data are plotted below the timeline. Left axis is for monkey mass plot (dotted line). Right axis gives percent change over baseline in striatal DVR (solid line).

### 5.2.3 Animal care procedures

To allow for accurate measurements of food intake, the animal was individually housed at the Wisconsin National Primate Research Center. Other monkeys were in adjacent cages as to minimize environmental effects. The room was maintained at a temperature of 21°C with a 12-hour light/dark cycle. The animal
was allowed *ad libitum* access to food for 8 hours/day starting at 8:00am and water was continuously available. The caloric intake and weight of the subject were recorded throughout the course of the experiments.

On the day of each scan the monkey was anesthetized with ketamine (15mg/kg) and transported from its home cage to the PET scanner. There was a period of greater than 50 minutes between administration of ketamine and the injection of radiotracer to minimize the potential effects on radioligand binding. Though the effects of ketamine on D₂/D₃ availability are small, approximately 2% (Nader et al 1999; Nader and Czoty 2008), this timing was recorded to examine potential confounding effects. Upon arrival at the PET scanner, the monkey was intubated and maintained under isoflurane at 0.75%-1.5% for the duration of the scan. The monkey was positioned face-down in a custom-made head-holder mounted to the scanner bed, yielding repositioning accuracy on the order of several millimeters between PET scans. Body temperature was maintained using a warm air heater and a continuous i.v. infusion of saline was administered to prevent dehydration. Heart rate, breathing rate, body temperature, and SpO₂ were monitored and logged during the course of each PET scan.

### 5.2.4 PET scans

¹⁸F-Fallypride was synthesized as described in Chapter 2. Following positioning, attenuation scans were acquired for 518 seconds using a Co-57 transmission point source. The dynamic emission PET scan was initiated with the 30 second bolus i.v. infusion of ¹⁸F-fallypride (5.12 ± 0.24 mCi, injected mass 0.05 ± 0.02 µg/kg) and data was acquired for 2.5 hours on a Concorde microPET P4 scanner (Tai et al 2001).
Emission data were acquired in list mode for the duration of the scan. Following the scan, the animal was removed from the anesthesia, allowed to recover, and returned to the housing facilities.

For the final PET scan, the stimulator was activated (130Hz, 2.0V) 110 minutes after the injection of $^{18}\text{F}\text{-fallypride}$ and the scan was continued for an additional 70 minutes. This procedure was followed to investigate the possible acute effects of stimulator activation. The stimulator was turned off immediately following the emission scan. Toggling power to the stimulators was performed via a transcutaneous DBS programmable device placed near the waveform generator, out of the field of view of the scanner. No motion in the animal was evident during the activation period.

### 5.2.5 Image processing

An attenuation sinogram was created using the segmented transmission scan. Emission listmode data for $^{18}\text{F}\text{-fallypride}$ were binned into 41 frames (frames x minutes per frame; 4x0.5, 5x1, 6x2, 5x3, 5x4, 15x6 ) making corrections for deadtime and randoms. The emission sinograms were reconstructed with filtered backprojection using a 0.3 cm$^{-1}$ Hann filter, zoom of 1.5x, and 128x128x63 voxel matrix size with voxel size of 1.26mm x1.26mm x1.21mm. Corrections were made for attenuation, decay, scanner normalization, and scatter to create images with quantitative units of nCi/cc.

$^{18}\text{F}\text{-Fallypride}$ binding was compared for all scans based on the measurement of the distribution volume ratio (DVR). Mathematically, DVR is described by the relationship $\text{DVR} = f_{ND}B_{\text{avail}}/K_D + 1$, which is equivalent to $\text{DVR} = \text{BP}_{ND} + 1$, where $B_{\text{avail}}$ is the density of receptors available for radioligand binding, $f_{ND}$ is the free fraction in the nondisplaceable compartment, $K_D$ is the apparent (in vivo) dissociation rate
constant, and $B_{ND}$ is the non-displaceable binding potential, as described previously (Innis et al 2007). Parametric images of DVR were created using the multi-linear reference tissue model described previously (Ichise et al 2003), using $t^* = 29$ minutes. The cerebellum was used as a reference region. Cerebellum time activity curves were obtained by drawing five 8.8mm-diameter circles on each of three slices on the posterior cerebellum. For the generation of voxel-based parametric images, each frame of the dynamic images was smoothed using a 3-voxel (3.8mm) FWHM 3D Gaussian kernel.

To account for the variability in positioning of the head holder, FSL flirt (Smith et al 2004) was used to register all images to a common space as defined by the pre-surgery MRI. This procedure involved the following steps: 1) registering each integrated PET scan to the T1-weighted MRI, 2) creating a PET template from an average of the coregistered PET images, and 3) registering each PET to the template image. Based on centroid matching in the striatum, the images were estimated to have sub-voxel alignment accuracy, which is expected for intrasubject, intramodal registrations (Woods et al 1998). The transformations were then applied to the $^{18}$F-fallypride DVR parametric images, putting all images (MRI, integrated PET and PET-DVR) into a common space, allowing for identical regions of interest (ROIs) to be applied to all images and facilitating voxel-based analyses. Similarly, to allow for comparison of time-activity curves, transformations and ROIs were applied to 4D dynamic PET data for both baseline and final scans.

ROIs were centrally placed within the boundaries of each region, defined on the MRI/PET coregistered images. The average value within each ROI is reported for DVR images as well as percent change from baseline. ROIs included (with volumes given) the left and right divisions of the caudate (0.16cc each), putamen (0.24cc each), substantia nigra (0.060cc), the inferior-medial region of the thalamus (thalamus,
0.030 cc), and the prefrontal cortex (0.967 cc). Percent change in DVR is reported relative to baseline: 
\[
\frac{\text{scan} - \text{baseline}}{\text{baseline}} \times 100\%.
\]
Voxel-based whole brain images of percent change from baseline DVR were created and examined for regional changes throughout the whole brain. Voxels with either the ‘scan’ or ‘baseline’ DVR value less than 1.0 were masked from these images. Assuming a constant ligand-receptor affinity, an increase in DVR (positive percent change) represents more available D2/D3 receptors, while a decrease in DVR (negative percent change) represents fewer available receptors.

To examine anterior-posterior changes within the striatum (investigated due to a visually apparent shift in binding in the 130Hz study) we examined line profiles (3 voxel width) through the striatum on a single axial slice of the DVR images.

The ROI data for the acute DBS activation (final) study was analyzed using a modified model to detect the presence of time-dependent changes in radiotracer binding due to the activation of the electrode. For this experiment, the method described by Alpert and colleagues (Alpert et al. 2003) was used to account for time-varying changes in the kinetic parameters as expressed in the equation:

\[
C(t) = RC_r(t) + k_2 \int_0^t C_r(u) du - k_2 \int_0^t C(u) du - \gamma T \int_T^t e^{-\gamma(u-T)} C(u) du,
\]

where \( C(t) \) and \( C_r(t) \) are the specific binding tissue region and reference region radioactivity concentrations, respectively, at time \( t \). \( R \) is the ratio of the delivery rate constants in the tissue and reference regions (\( K_t/K_{tr} \)), \( k_2 \) is the tissue to plasma efflux constant in the tissue region. The \( \gamma \) term represents the temporal change in the \( k_2/DVR \) parameter. The presence of endogenous neurotransmitter
competition with the radioligand at the receptor sites would be reflected by a temporal change in DVR and is accounted for in the model with the $e^{-\tau(t-T)}$ term, at some time $T$. For this experiment, we have chosen $T$ at the time of stimulator activation. The decay constant $\tau$ describes the rate at which this temporal variation discontinues and returns to baseline. Because the stimulator was activated for the remainder of the PET experiment, a value of $\tau=0$ was chosen, thus keeping this term constant (rather than a decaying exponential). The baseline period (110 minutes) of the PET experiment provides sufficient data for estimating the parameters $R$, $k_2$ and $k_2/DVR$ and the post-activation data (110 – 180 minutes) yields information for measuring $\gamma$. An increase in competing endogenous dopamine would result in $\gamma>0$ whereas a decrease would result in $\gamma<0$. Significance of the $\gamma$ parameter was based upon the t-statistic calculated as $\gamma/\sigma_\gamma$; a threshold of $p<0.05$ was selected as significant.

To serve as a comparison with the other PET scans, parametric images of $^{18}$F-fallypride DVR were also created from this study based on the post hoc assumption that no DBS induced change in binding occurred (i.e. null hypothesis that $\gamma=0$). For this calculation, the identical model as used for the other PET scans was applied, using only the first 2.5 hours of dynamic data for the estimation of DVR.

### 5.3 Results

During the course of the experiment there was an increase in the weight of the animal. The initial weight was 14.8 kg and final weight was 16.3 kg. Figure 5-2b shows the time course of weight gain during the experiments. Pre-surgery baseline average daily caloric intake was 431 kcal/day. During stimulation periods daily caloric intake was 677 kcal/day (130Hz) and 515 kcal/day (50Hz). Washout and post-stimulation period averages were 579 kcal/day and 575 kcal/day, respectively.
The change in striatal $^{18}$F-fallypride DVR in relation to weight gain is shown in Figure 5-2b. ROI analyses revealed significant increases in DVR in all regions during both high and low frequency stimulation (Figure 5-3). The striatal regions showed large increases while the extrastriatal regions showed modest to large increases during stimulation periods. For both washout and final scans (stimulators off), DVR values returned to near baseline DVR values; with the exception of the substantia nigra during the washout period (-17%) and both the PFC and thalamus post-stimulation (~20%). Comparisons of the left and right striatal regions revealed no significant asymmetries in DVR due to the unilateral stimulation.

A closer examination of the striatal region suggests a re-distribution of available receptors within the striatum (Figure 5-4). For 130 Hz stimulation, increases in DVR were greater in the anterior striatum than posterior, especially on the left side, resulting in a visually apparent shift of the region with highest DVR. The shift in binding is also illustrated in Figure 5-5, where the long-axis profile reveals a bimodal shape for the 130Hz scan.

The isocontours on Figure 5-4 reveal the same pattern as seen in the ROI analysis of the caudate and putamen, with an increase in $^{18}$F-fallypride DVR for both stimulation scans and a return to baseline DVR values during both the washout and post-stimulation periods. Figure 5-6 highlights DBS induced increases in DVR in the substantia nigra region; showing a similar trend as seen in the striatum with changes most profound during the stimulation scans.
Figure 5-3: ROI analysis

The regional changes in DVR over the course of the experiment. The bar graphs represent change in average DVR value within a ROI from baseline to the experimental scans ([scan-baseline]/baseline)x100%. Values on top of graph represent baseline DVR value. PFC = prefrontal cortex, SN = substantia nigra, thal = thalamus.
Figure 5-4: Parametric image results (striatum)

Transaxial slices through the striatum as indicated in the midsagittal MRI (bottom left). In the top row, the color overlay indicates parametric images of $^{18}$F-fallypride DVR (thresholded to include only striatum). The percent change in DVR (relative to baseline) is shown in the bottom row. Voxels with DVR<1 in either ‘baseline’ or ‘scan’ were masked from the image. White dotted lines show the outline of the putamen ROI.
Figure 5-5: Striatal line profiles

Striatal profiles showing redistribution of $^{18}$F-fallypride binding during 130Hz stimulation. Profile lines are 3 voxels wide, as drawn on an axial slice 3mm superior to those of Figure 5-4. The x-axis represents position along profile line, with zero representing the lower portion of the profiles.
Figure 5-6: Parametric Image Results (sub. nigra)

Transaxial slices through the region of the substantia nigra (white arrow) as indicated by the midsagittal MRI (bottom, left). In the top row, the color overlay indicates parametric images of $^{18}$F-fallypride DVR (thresholded to accentuate binding in the substantia nigra). The percent change in DVR (relative to baseline) is shown in the bottom row. Voxels with DVR<1 in either ‘baseline’ or ‘scan’ were masked from the image. White dotted lines show the outline of the substantia nigra ROI.
Figure 5-7: TACs for post-stimulation study

Time-activity curves through the putamen (triangles), caudate (squares) and substantia nigra (circles) for both the baseline scan (filled points) and the final scan (open points). The DBS electrode was activated at 110 minutes, as indicated by the vertical dotted line.
Time-activity curves of $^{18}$F-fallypride in the caudate, putamen and substantia nigra for both baseline and the final scan are shown in Figure 5-7. Kinetic analysis with the time dependent term revealed no significance in the $\gamma$ parameter, suggesting that acute changes in $^{18}$F-fallypride binding are not present.

### 5.4 Discussion

As the clinical applications of deep brain stimulation target regions in the basal ganglia, in which the dopaminergic system in an integral part, deep brain stimulation lends itself well to dopamine receptor neuroimaging applications. This work was performed in collaboration with Dr. P. Charles Garell, a former neurosurgeon at the UW Hospital, who had performed the DBS surgery on this monkey in an experimental treatment for obesity by stimulating the bed nucleus of the stria terminalis. Considering the functional connections between the BNST and dopaminergic neurons, the implicated role of dopamine in obesity and feeding patterns (Wang et al 2002), and our experience with dopaminergic imaging; we had a unique opportunity to participate in a novel experiment investigating the effects of DBS on the dopamine system in an healthy research subject. $^{18}$F-Fallypride was used as the PET radioligand due to its high selectivity for the D$_2$/D$_3$ receptors, favorable binding characteristics for measuring striatal and extrastriatal binding, and its suitability for translation to human studies. Considering the relatively non-invasive nature of the scanning protocol and that the stimulation parameters used in this experiment are similar to those used clinically, it is within reason that the methods used in this study could also be applied to humans with DBS electrodes in regions other than the BNST. Furthermore, this proves to be a novel application of a high-affinity radioligand, showing high sensitivity to detecting a physiological change with functional imaging.
Throughout both the striatal and extrastrial regions of the brain we report large changes in \( ^{18}\text{F}-\text{fallypride} \) binding resulting from chronic simulation of the BNST. Because these studies were performed in a single animal, it is not possible to report statistical significance to the measured changes. However, comparison to intrasubject test-retest variability of 10% with \( ^{18}\text{F}-\text{fallypride} \) DVR in non-human primates (Christian et al 2000) suggests that reported DVR changes in excess of 20% have a high likelihood of being due to the effects of DBS.

In research applications with reversibly bound PET neuroligands such as this where DVR is the metric measured, a change in DVR can be interpreted as (i) a change in the number of available receptors, \( B_{\text{avail}} \), (ii) a change in the apparent dissociation rate constant (\( K_D \)) via a change in the concentration of competing endogenous neurotransmitter, or (iii) a combination of both (for review see (Laruelle et al 1997)). The nature of DBS in modulating neuronal firing combined with previous in vivo microdialysis work showing DBS modulation of neurotransmitter systems provides evidence that changes in DVR likely reflect alterations in competing endogenous neurotransmitter concentration (McIntyre et al 2004).

Along the line of the highly variable, small changes we saw in the previous chapter due to dopamine depletion via AMPT, we look at the potential decreases in endogenous dopamine caused by DBS induced inhibition of downstream circuits. A reduction in endogenous dopamine would produce more radiotracer binding and a positive change in DVR. Using pharmacological induced dopamine depletion with reserpine, increases in nondisplaceable binding potential of 30% - 50% have been reported in nonhuman primates (Dewey et al 1992; Ginovart et al 1997), serving as an upper limit to changes in DVR due to competing endogenous dopamine. Because the results we observed were of this magnitude, we hypothesize that the reported changes are due to reduced dopamine competition, rather than an increase in
the number of receptors. However, this question could be addressed by either invasively measuring dopamine concentration with a microdialysis probe or by using a multiple-injection protocol (see Chapter 3).

Despite coming from only one animal, the changes in D<sub>2</sub>/D<sub>3</sub> binding were profound, so we speculate on the cause of the observed changes. The alterations in the D<sub>2</sub>/D<sub>3</sub> binding are likely to be caused by stimulation of the BNST, which has an influence over a variety of dopaminergic neurons, including a high density of projections to the substantia nigra (Fudge and Haber 2001). If these projections were inhibited by the DBS, dopamine production of nigro-striatal neurons could have been shut down, leading to a decrease in striatal dopamine and the observed increases in DVR. Despite the large apparent decrease in striatal dopamine, the monkey did not show any change in control of movements. In the substantia nigra, the smaller change in DVR could be the result of being under direct influence from the stimulated neurons. It is also possible that the D<sub>2</sub>/D<sub>3</sub> autoreceptors in the substantia nigra (Diana and Tepper 2002) are not as sensitive to endogenous dopamine as synaptic receptors in the other regions. Since DBS cannot deliver a uniform electric field across the entire target nucleus, different sub regions of the BNST may have been stimulated to different degrees (McIntyre et al 2004). This differential stimulation pattern may have been relayed through the substantia nigra to the striatum, leading to the observed change in striatal binding distribution during 130Hz stimulation. It should not be expected that changes would be constrained to only the regions under direct stimulation or within 1-2 synapses of stimulated neurons, but also to any regions that are part of a larger neural circuit containing the nuclei or axons under direct stimulation (Montgomery and Gale 2008). These higher degree connections may be responsible for the changes seen in regions such as the prefrontal cortex and the thalamus.
The final study did not detect any significant acute changes in $^{18}$F-fallypride binding due to changes in endogenous dopamine. Visual inspection of the caudate and putamen time activity curves (Figure 5-7) shows a subtle departure from the corresponding baseline data beginning at the DBS activation. However, the time dependent term, $\gamma$, in the kinetic model could not sufficiently separate changes in specific binding from changes in radioligand delivery (via blood flow) due to the high correlation between parameters (Alpert et al 2003). While the methods used here did not detect a significant change, the prospects of measuring acute changes in dopamine release induced by DBS remain intriguing and warrant further investigation using methods with improved detection sensitivity, possibly characterizing both changes in magnitude and release timing (Morris et al 2008).

Previous studies have looked at striatal binding potential in both stimulator-ON and stimulator-OFF scans (Abosch et al 2003; Hilker et al 2003; Strafella et al 2003). In these studies, subjects had DBS probes implanted in the subthalamic nucleus for treatment of Parkinson’s Disease. Although these patients had PD, the experimenters hypothesized that DBS might restore normal dopaminergic function in the striatum, reducing the measured binding. With no change in binding potential detected between states, the authors all conclude that DBS does not restore normal dopaminergic activity in the striatum. The lack of observed changes in binding potential between ON and OFF scans points to the limitations in conducting such experiments in subjects with severely degenerated nigro-striatal innervation and with limited ability to evoke a dopamine response. In the study we performed; however, all neurons under the influence of DBS are assumed to be healthy, functioning neurons with the capacity to modulate dopamine release, yielding the potential for measuring large changes in $^{18}$F-fallypride binding, as we observed. Furthermore, by choosing a high-affinity D$_2$/D$_3$ antagonist for the radiotracer, we were able to examine regions outside the D$_2$/D$_3$ receptor rich striatum, where the effects of DBS may also play a prominent role in the clinical effectiveness.
Also of considerable interest is the positive correlation of striatal DVR with weight gain. While the monkey did gain weight over the period of the whole study, it was during the stimulation periods that most of the weight was gained. This was also the period of the largest increases in DVR. There was less weight gain during the washout and post-stimulation periods, both of which correspond to a return of DVR to baseline values. This is in contrast to a previous study that showed a negative correlation between body mass index (an index of obesity) and $^{11}$C-raclopride binding potential (Wang et al 2001). Our results are more in line with previous findings that report a normalization of weight in obese mice following treatment with a dopamine D$_1$/D$_2$ agonist, SKF-38393 (Bina and Cincotta 2000). With these conflicting results, we can only suggest that dopamine may play a role in weight gain, and suggest that further examination of how differences in receptor density and differences in synaptic dopamine individually contribute to feeding patterns and body mass.

With PET having now shown great promise in DBS research, we can suggest a wide range of additional studies on DBS animals to provide a further understanding of how DBS changes the D$_2$/D$_3$ receptor system. These include measurement at a variety of stimulation amplitudes and over a wider range of stimulation frequencies. Also of great interest would be a correlation of behavioral data with temporal changes in D$_2$/D$_3$ receptor binding following the initiation of simulation and after its termination, possibly providing insight into the receptor dependent thresholds of DBS therapeutic effectiveness. Since there are an abundance of clinical subjects with DBS, it is more likely that the future of PET neuroreceptor imaging in DBS will be focused on human subjects as opposed to in animal models. This will lead to challenges in data interpretation due to the fact that humans with DBS electrodes are being treated for a disease, for there will never be “normal controls” in human DBS research due to the invasiveness of the surgery. Even so, PET would aid in the understanding of changes at dopaminergic synapses during DBS which could
lead to more effective clinical uses of DBS or as an inspiration for new experimental applications for DBS.

5.5 Conclusion

PET neuroligand imaging using \(^{18}\text{F-}\text{fallypride}\) has demonstrated the sensitivity to track changes in dopamine D\(_2\)/D\(_3\) binding during the course of deep brain stimulation. The results show a profound change in \(^{18}\text{F-}\text{fallypride}\) binding due to stimulation of both 130Hz and 50Hz and a return to baseline DVR values when the stimulator was turned off during both washout and after stimulation. This work is the first to use a high-affinity radioligand for imaging a DBS subject. These methods show great potential for providing insight into the neurochemical mechanisms of DBS, and warrant further use of PET imaging with high affinity radioligands in deep brain stimulation research.
5.6 References


Chapter 6

Conclusions

The experiments presented in this dissertation were motivated by the need for the scientific community to have a better understanding of the extrastriatal dopamine system. This need may be realized through the advancement of PET imaging methods used to study dopamine receptors. This greater knowledge of the dopamine system will not only satisfy our curiosity of how the brain works, but it may lead to a better understanding of pathologies of dopamine system and development of advanced methods for treating patients with neuropsychiatric pathologies. After first developing the tools needed to synthesize both tracers, we were able to use $^{11}$C-FLB457 and $^{18}$F/$^{11}$C-fallypride as tools to probe into functions of the dopamine system. The \textit{in vivo} characteristics of both radioligands were evaluated in baseline conditions and in conditions where the degree of binding was altered via either pharmacological and electrophysiological manipulations. The comparisons we made were performed in the same animals using similar methods for both tracers, providing a more valuable comparison than could be made based on results found in the literature from experiments performed over a variety of species using different measurement techniques.

The multiple injection technique was used to fully characterize the \textit{in vivo} kinetics of both $^{18}$F-fallypride and $^{11}$C-FLB457. While multiple-injection experiments are technically complicated; their use was the appropriate method for measurement of the rate constants associated with both vascular transport and specific binding. Other imaging experiments used single-injection experiments in order to evaluate how measurements of drug occupancy compare when using $^{11}$C-FLB457 and $^{11}$C-fallypride, a type of measurement that can be important for establishing proper dosing of antipsychotic medications.
Finally, we performed a set of experiments in a monkey with chronic deep brain stimulation, demonstrating the utility of high-affinity PET radioligands for investigations of changes in neuroreceptor availability during electrophysiological treatments.

Based on the results of the experiments we performed we find that in experiments were both striatal and extrastriatal measurements are desired, $^{18}$F-fallypride is the most appropriate radioligand to use due to its much longer half-life than either $^{11}$C-FLB457 or $^{11}$C-fallypride. $^{18}$F/$^{11}$C-Fallypride would be best suited for displacement studies, due to its rapid clearance from free space. Both $^{11}$C-FLB457 and $^{11}$C-fallypride are suited for assay of extrastriatal D$_2$/D$_3$ receptors, but the combination of FLB457’s higher affinity and slower $k_2$ give it higher count rates in late time frames, giving it an advantage of having a better signal to noise ratio. For investigators primarily interested in cortical D$_2$/D$_3$ receptor quantification, we recommend the use of arterial blood sampling methods as to eliminate the potential for bias introduced by specific binding in the reference region. The methods used in these experiments should serve as a template for evaluation new radioligands that are developed for assay of cortical D$_2$/D$_3$ binding.

These experiments provide a initial comparison of fallypride and FLB457, but in each set of experiments, time and resources were limited and improvements to the methods should be considered. In our study, a small number of monkeys were used; two for multiple injection studies, three for drug manipulation studies, and only one for the DBS. The results would be strengthened if the studies could be repeated in a larger number of animals. Specifically, proper identification of binding parameters of FLB457 in multiple-injection experiments was only achieved in one experiment, so repeated MI studies with FLB457 would provide further confirmation of our results. For the drug manipulation studies, we did not measure the arterial input function due to the demands on personnel for preparing multiple doses of $^{11}$C-labeled
radiotracers. If these measurements were made; however, we would more accurately measured the degree of true drug occupancy by eliminating the bias associated with the using the cerebellum as a reference region. Furthermore, comparisons of tracers after dopamine depletion could be improved with a more robust method for dopamine depletion, such as using repeated oral administration of AMPT, as is done in human studies, or by administration of reserpine. Finally, as we conclude that methods using the cerebellum as a reference region are subject to a bias in binding potential, it would be of great interest to develop a simple method for determining the magnitude of cerebellar binding so that the bias can be better quantified or perhaps a large cohort measurement of cerebellar $D_2/D_3$ receptor density.

PET assay of extrastriatal dopamine receptors could lead to a better scientific understanding of how the dopamine system works and the mechanisms behind disorders of this system. This work has provided specific details of how both $^{11}$C-FLB457 and $^{18}$F/$^{11}$C-fallypride behave in vivo, as well a descriptions of how these measurements were made during baseline conditions and after pharmacological and electrophysiological manipulations of radioligand binding. These details may be of immediate interest to researchers involved in kinetic modeling of radioligands used in neuroimaging, but the impact of their conclusions has the potential to be more far-reaching. With greater knowledge of radioligand characteristics, functional imaging methods can be improved, and studies can be better designed with greater sensitivity to biological changes. With greater sensitivity to biological changes, the scope of what types of experiments can be performed may be broadened, leading to a better understanding of dopamine function which implications in both clinical applications and neuropsychiatric research. A better understanding of disease states may enable clinicians to be able to provide more effective treatments of disorders such as schizophrenia or drug addiction. Furthermore, the development of neuroimaging techniques to study the underlying neurochemical methods of deep brain stimulation may someday provide a better theory as to how DBS works, allowing more treatment options for patients with
movement disorders or the development of treatments of neurological disorders that currently have few treatment options.