Molecular imaging of striatal and extrastriatal components of the dopamine system: Positron emission tomographic studies in healthy subjects and Parkinson Disease

Submitted for the degree of:

Doctor of Philosophy

Swinburne University of Technology

2008

Vanessa Louise Cropley
Brain Sciences Institute
Faculty of Life and Social Sciences
Swinburne University of Technology
Melbourne
Australia
Abstract

Dopamine plays a pivotal role in the regulation and control of cognition, movement and motivation and is involved in a variety of neurological and psychiatric disorders. Molecular imaging with positron emission tomography (PET) and radiolabeled compounds has enabled the in vivo assessment of the distribution and density of receptors, enzymes and other cellular processes pertaining to the dopamine system in normal and pathological states. Until recently, such measurements have been restricted to the striatum, due to the paucity of appropriate radioligands for measurement of low density targets. With the development of radioligands suitable for extrastriatal measurement, examination of the different components of the dopamine system in not only the striatum, but also extrastriatal regions, is possible, in living human brain.

Therefore, the aims of this thesis were to assess, by means of two independent experimental PET studies, the feasibility of imaging pre-, post- and intra-synaptic components of the dopamine system in the striatum, as well as extrastriatal areas. Change in dopamine markers were assessed in human subjects with a compromised dopamine system, altered by pharmacological (Study 1) or pathological (Study 2) means. Specifically, intra-synaptic dopamine transmission (“phasic” and “tonic” dopamine release) was examined with $^{18}$F-fallypride (for post-synaptic D$_2$-like receptors) and pharmacological challenges to either increase or decrease dopamine transmission in young healthy subjects (Study 1), whereas pre- and post-synaptic dopamine transmission was assessed with $^{18}$F-FDOPA (for pre-synaptic dopamine synthesis) and $^{11}$C-NNC 112 (for post-synaptic D$_1$-like receptors) in a patient cohort characterised by chronic dopamine deficiency (Parkinson disease) (Study 2). In each study, associations between these dopamine markers and higher cognitive functions were explored.

Findings confirm that $^{18}$F-fallypride and $^{11}$C-NNC 112 are able to be quantified in striatal and extrastriatal regions. Cortical $^{18}$F-FDOPA uptake however, was unable to be quantified. Stimulant-induced dopamine release with d-amphetamine successfully displaced $^{18}$F-fallypride binding in striatum and several extrastriatal regions. However, depletion of dopamine with AMPT did not appear to modulate $^{18}$F-fallypride in any region (Study 1). Parkinson disease patients did not show alteration of striatal or cortical
D₁ receptors despite significantly reduced dopamine metabolism in the striatum, which was associated with executive impairment (Study 2).

These studies demonstrate the capability of PET, with radioligands targeting pre-synaptic synthesis and post-synaptic D₁ and D₂ receptors, to assess different components of the dopamine system in striatum and extrastriatum. The pitfalls and complexity of radioligand imaging are discussed within the context of [¹⁸F]fallypride, [¹¹C]NNC 112 and [¹⁸F]FDOPA measurement. Lastly, this thesis demonstrates some promise for using molecular imaging to explore the neurochemical underpinnings of higher cortical function.
Declaration

I declare that this thesis does not incorporate, without written acknowledgement, any material that has previously been submitted for the award of any other degree or diploma in any university, college, or other educational institution; and to the best of my knowledge, this thesis does not contain any material previously published or written by another person except where due reference is made in the text, including the disclosure of contributions for any work based in joint research or publications.

I declare that the ethical principles and procedures specified in the Swinburne University of Technology Human Research Ethics document on human research and experimentation have been adhered to in the presentation of this thesis.

Name: Vanessa Louise Cropley

May, 2008
Acknowledgements

I would first like to thank my supervisors for their guidance, support and expertise throughout this thesis. Specifically, I thank my coordinating supervisor, Associate Professor Pradeep Nathan, for encouraging me to do a PhD, and for his mentorship, ideas and tireless enthusiasm over the course of this thesis. I am extremely grateful to Professor Robert Innis for allowing me to work in his laboratory at the National Institutes of Health. I feel very fortunate to have worked with a researcher and within a research group of such high caliber and sincerely thank Professor Innis for his expertise, mentorship and role he has played in my academic development. I extend my sincere gratitude to Dr. Masahiro Fujita for his tremendous contribution and input to this thesis work. Dr. Fujita taught me the skills needed to conduct and analyze molecular imaging data; his knowledge, patience, time and effort have been invaluable and to which I am extremely grateful. Thank you.

The studies presented in this thesis were conducted at the National Institutes of Health (Bethesda). There are many individuals that have helped make these studies possible, who are acknowledged in the published work (see appendices). Special thanks go to Dr. Victor Pike and the radiochemists for radioligand preparation and synthesis; Janet Sangare, Dr. William Bara-Jimenez, Dr. Alicja Lerner and Dr. Xiang-Yang Zhang for clinical support; Dr. Amira Brown for neuropsychological support; Dr. Jeih-San Liow for technical support, and Robert Gladding and the staff of the NIH PET Department and NIH Clinical Center Nursing Unit for successful completion of the scans. I also extend my thanks to all members of the Molecular Imaging Branch for their friendship and support. It was a pleasure working within such a multi-cultural, skilled and dedicated team, who have all been an inspiration to me and helped make my time abroad both positive and memorable.

Finally, I would like to thank my family and friends. To my friends, both here in Melbourne and abroad, I can’t thank you enough for your friendship, interest and encouragement. Especially, thank you to Sarsha, Kate, Roshni, Cath and Tom, and Kalyan, for always believing in me. And lastly, to my family – Mum, Dad, Rebekah and Travis, as well as extended family, thanks for your continual support and patience throughout my studies including these PhD years.
List of Tables

Chapter 1
Table 1-1 Common radioligands for PET and SPECT imaging of the dopamine system .................................................................18
Table 1-2 PET and SPECT studies of intra-synaptic dopamine transmission in healthy humans using pharmacological challenges ........................................45
Table 1-3 PET and SPECT studies of intra-synaptic dopamine transmission in clinical populations compared to controls using pharmacological challenges ........................................................................................................50

Chapter 2
Table 2-1 Molecular imaging studies showing associations between presynaptic dopamine and cognition in healthy subjects and patient populations ....68
Table 2-2 Molecular imaging studies showing associations between postsynaptic dopamine receptors and cognition in healthy subjects and patient populations ........................................................................75
Table 2-3 Molecular imaging studies showing associations between dopamine release and cognition in healthy human subjects .........................77

Chapter 4
Table 4-1 Properties of some positron emitting radioisotopes .........................87
Table 4-2 Spatial, temporal and sensitivity parameters associated with PET and MR technology ...............................................................................89

Chapter 5
Table 5-1 Participant demographics and clinical measures ..............................123
Table 5-2 Test-retest reproducibility of measuring [18F]fallypride binding potential (BPND) ..............................................................................130
Table 5-3 Test-retest reproducibility of measuring [18F]fallypride binding potential (BPND) in striatal subdivisions ..............................................130
Table 5-4 Amphetamine-induced changes in [18F]fallypride binding .............131
Table 5-5 Amphetamine-induced changes in [18F]fallypride binding in striatal subdivisions ................................................................................132

Chapter 6
Table 6-1 Participant demographics and clinical measures ..............................143
Table 6-2 Striatal [18F]FDOPA influx constant in PD patients compared to
controls .............................................................................................................. 148

Table 6-3 Effect of partial volume correction on frontal $^{[18}\text{F}]$FDOPA influx constants in Parkinson disease patients .............................................. 150

Table 6-4 $^{[11}\text{C}]$NNC 112 binding potential from region of interest analysis ..........151
List of Figures

Chapter 1

Figure 1-1  The major brain dopamine projections. Figure reproduced from Szabo et al (2000) ................................................................. 5
Figure 1-2  Schematic diagram of a typical dopamine synapse and neuron illustrating the synthesis, release, uptake and metabolism of dopamine .... 7
Figure 1-3  Pathways for the metabolism of dopamine in the brain. Diagram reproduced from Volkow et al (1996b) ...................................................... 8
Figure 1-4  Simplified diagram of the dopamine receptor signalling pathways in the brain. Figure reproduced from Meyer and Quenzer (2005) .......... 9
Figure 1-5  Radioligand target sites and examples of radioligands used for imaging the dopaminergic system with PET and SPECT ......................... 15
Figure 1-6  Schematic diagram of the fronto-striato-thalamic circuits. Figure reproduced from Kaasinen and Rinne (2002) ................................. 17
Figure 1-7  Simplified graphical representation of the metabolism of $[^{18}\text{F}]$FDOPA in the periphery and brain ................................................................. 21
Figure 1-8  Simplified diagram of the classic occupancy model. Figure reproduced from Laruelle (2000) ................................................................. 41
Figure 1-9  Simplified illustration of the internalisation model. Figure reproduced from Laruelle (2000) ................................................................. 55

Chapter 3

Figure 3-1  Structure of $[^{18}\text{F}]$fallypride ....................................................... 83
Figure 3-2  Structure of $[^{18}\text{F}]$FDOPA ......................................................... 84
Figure 3-3  Structure of $[^{11}\text{C}]$NNC 112 ..................................................... 84

Chapter 4

Figure 4-1  Components of PET imaging .................................................... 86
Figure 4-2  Positron detection with PET ...................................................... 88
Figure 4-3  Three compartment model used to describe in vivo receptor radioligand kinetics in brain ................................................................. 99
Figure 4-4  Schematic diagram of the steps involved in MRI-based partial volume correction of PET activity data ............................................. 115

Chapter 5

Figure 5-1  Parametric image of $[^{18}\text{F}]$fallypride $BP_{ND}$ in a healthy subject 129
Chapter 6

Figure 6-1  Mean parametric image of $^{18}$F-FDOPA $K_i$ of healthy subjects superimposed onto MRI template (a). $K_i$ values are greater in cortical white matter than adjacent gray matter regions (b). The kinetics of white matter uptake and washout from a representative healthy subject is significantly different from reference region occipital cortex (c) .......... 149

Figure 6-2  Mean parametric image of $^{11}$C-NNC $112 B_{PD}$ of healthy subjects ...... 151

Figure 6-3  Wisconsin card sorting test categories achieved score versus $^{18}$F-FDOPA uptake ($K_i$) in the putamen of PD patients ......................... 152
List of Abbreviations

3-MT     3-methoxytyramine
3-OMFD    3-O-methyl-[18F]FDOPA
5-HT      Serotonin
6-[18F]FDOPA   6-[18F]fluoro-L-3,4-dihydroxy-phenylalanine
AADC     Aromatic Amino Acid Decarboxylase
AC       Adenylyl Cyclase
AC-PC    Anterior Commissure – Posterior Commissure
AD       Aldehyde Dehydrogenase
ATP      Adenosine Triphosphate
AMPT     α-methyl-para-tyrosine
ANOVA    Analysis of Variance
BBB      Blood-Brain Barrier
BH4      6-tetrahydrobiopterin
BPND     Binding Potential
cAMP     Cyclic Adenosine Monophosphate
COMT     Catechol-O-methyltransferase
COWAT    Controlled Oral Word Association Test
CSF      Cerebrospinal Fluid
DAT      Dopamine Transporter
DLPFC    Dorsolateral Prefrontal Cortex
DOPAC    Dihydroxyphenylacetic Acid
[18F]FMT   [18F]Fluoro-m-tyrosine
FDA      Fluorodopamine
FDOPAC   Fluoro-dihydroxyphenylacetic acid
FDR      False Discovery Rate
FHVA     Fluoro-homovanillic acid
FLIRT    FMRIB’s Linear Image Registration Tool
FWHM    Full-Width at Half Maximum
HPLC    High Performance Liquid Chromatography
HVA     Homovanillic Acid
ICC      Intraclass Correlation Coefficient
L-dopa   L-3,4-dihydroxyphenylalanine
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAO</td>
<td>Monoamine Oxidase</td>
</tr>
<tr>
<td>MNI</td>
<td>Montreal Neurological Institute</td>
</tr>
<tr>
<td>MPTP</td>
<td>1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>MRTM2</td>
<td>Multilinear Reference Tissue Model 2</td>
</tr>
<tr>
<td>NIH</td>
<td>National Institutes of Health</td>
</tr>
<tr>
<td>$[^{11}\text{C}]{\text{NMSP}}$</td>
<td>$[^{14}\text{C}]{\text{N-methylspiperone}}$</td>
</tr>
<tr>
<td>NMDA</td>
<td>$N$-methyl-$D$-aspartate</td>
</tr>
<tr>
<td>PET</td>
<td>Positron Emission Tomography</td>
</tr>
<tr>
<td>PD</td>
<td>Parkinson Disease</td>
</tr>
<tr>
<td>PFC</td>
<td>Prefrontal Cortex</td>
</tr>
<tr>
<td>PMOD</td>
<td>Pixel-wise Modeling</td>
</tr>
<tr>
<td>POMS</td>
<td>Profile of Mood States</td>
</tr>
<tr>
<td>PVC</td>
<td>Partial Volume Correction</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of Interest</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SDMT</td>
<td>Symbol Digit Modality Test</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard Error of the Mean</td>
</tr>
<tr>
<td>SPECT</td>
<td>Single Photon Emission Computed Tomography</td>
</tr>
<tr>
<td>SPM</td>
<td>Statistical Parametric Mapping</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for the Social Sciences</td>
</tr>
<tr>
<td>SRTM</td>
<td>Simplified Reference Tissue Model</td>
</tr>
<tr>
<td>TH</td>
<td>Tyrosine Hydroxylase</td>
</tr>
<tr>
<td>TPD</td>
<td>Tyrosine and Phenylalanine Depletion</td>
</tr>
<tr>
<td>UPDRS</td>
<td>Unified Parkinson’s Disease Rating Scale</td>
</tr>
<tr>
<td>VAS</td>
<td>Visual Analog Scale</td>
</tr>
<tr>
<td>VMAT2</td>
<td>Vesicular Monoamine Transporter 2</td>
</tr>
<tr>
<td>WCST</td>
<td>Wisconsin Card Sorting Test</td>
</tr>
</tbody>
</table>
Table of Contents

Abstract ......................................................................................................................................... i
Declaration .................................................................................................................................. iii
Acknowledgements .................................................................................................................... iv
List of tables ................................................................................................................................ v
List of figures ............................................................................................................................. vii
List of abbreviations .................................................................................................................. ix

THESIS OVERVIEW ....................................................................................................................1

1 INTRODUCTION: MOLECULAR IMAGING OF THE DOPAMINE SYSTEM ..........3

1.1 THE DOPAMINE SYSTEM........................................................................................3
   1.1.1 Introduction and history ...............................................................................3
   1.1.2 Dopaminergic pathways ............................................................................4
   1.1.3 Synthesis and release of dopamine .............................................................6
   1.1.4 Dopamine termination: reuptake and metabolism ......................................7
   1.1.5 Dopamine receptors ..................................................................................8
   1.1.6 Regulation of dopamine release: phasic and tonic modes of dopamine
       transmission ..........................................................................................................11

1.2 ASSESSMENT OF THE DOPAMINE SYSTEM: MOLECULAR IMAGING ......12
   1.2.1 Assessment of pre-, intra- and post-synaptic components of the dopamine
       system ......................................................................................................................14
   1.2.2 Examination of striatal and extrastriatal regions ........................................15
   1.2.3 Examination of smaller brain regions and subdivisions and subsequent
       examination of the frontostriatal circuitry ......................................................16
   1.2.4 Multi-tracer imaging approach to the examination of dopaminergic mechanisms 18

1.3 MOLECULAR IMAGING OF THE DOPAMINE SYSTEM IN THE HUMAN
       BRAIN: A SELECTED REVIEW............................................................................19
   1.3.1 Assessment of pre-synaptic dopamine function ..........................................20
   1.3.2 Assessment of post-synaptic dopamine receptors .......................................29
   1.3.3 Assessment of intra-synaptic dopamine release ..........................................39

2 MOLECULAR IMAGING OF THE DOPAMINE SYSTEM AND ITS ASSOCIATION
       WITH HUMAN COGNITIVE FUNCTION ..........................................................57
   2.1 INTRODUCTION AND BACKGROUND.............................................................57
      2.1.1 Dopamine and cognition: key animal and human studies .......................57
      2.1.2 Using molecular imaging to explore the relationship between dopamine and
          human cognition .................................................................................................61
   2.2 PRE-SYNAPTIC DOPAMINE MODULATION OF COGNITIVE FUNCTION ....62
2.2.1 Striatal $^{18}$FFDOPA studies in Parkinson disease ................................................. 62
2.2.2 Extrastriatal $^{18}$FFDOPA studies in Parkinson disease ................................................. 64
2.2.3 DAT studies in Parkinson disease .............................................................................. 65
2.2.4 DAT studies in healthy aging ..................................................................................... 66
2.2.5 Overview of pre-synaptic dopaminergic markers and cognitive function ............. 70

2.3 DOPAMINE RECEPTORS AND COGNITIVE FUNCTION ................................... 71
2.3.1 Dopamine D$_2$ receptors ......................................................................................... 71
2.3.2 Dopamine D$_1$ receptors ......................................................................................... 73

2.4 DOPAMINE RELEASE AND COGNITIVE FUNCTION ........................................ 76

2.5 SUMMARY ............................................................................................................... 79

3 RATIONALE OF THESIS ................................................................................................... 81
3.1 GENERAL THESIS OBJECTIVES AND AIMS ....................................................... 81
3.2 SPECIFIC AIMS OF EXPERIMENTAL STUDIES .................................................. 81
3.3 RADIOLIGAND SELECTION .................................................................................... 83
3.3.1 $^{18}$F]Fallypride ........................................................................................................ 83
3.3.2 $^{18}$F]FDOPA .............................................................................................................. 83
3.3.3 $^{11}$C]NNC 112 ............................................................................................................ 84

4 GENERAL METHODS AND PRINCIPLES ..................................................................... 85
4.1 PRINCIPLES AND CHARACTERISTICS OF POSITRON EMISSION TOMOGRAPHY ..................................................................................................................... 85
4.1.1 Basis of PET imaging .............................................................................................. 85
4.1.2 Spatial, temporal and sensitivity parameters of PET technology ......................... 89
4.1.3 Positron detection and system performance: effects on image quality and quantitation .......................................................................................................................... 90
4.2 OVERVIEW OF PET RADIOLIGAND DEVELOPMENT AND CRITERIA ..... 93
4.3 KINETIC MODELING AND QUANTIFICATION OF PET RADIOLIGANDS: REFERENCE TISSUE MODELS ...................................................................................... 96
4.3.1 Reversible versus irreversible ligands ...................................................................... 97
4.3.2 Compartmental versus graphical models ............................................................... 98
4.3.3 Use of reference tissue in PET quantification: Reference tissue models .......... 101
4.3.4 Reference tissue models used in the current thesis .............................................. 102
4.4 METHODS FOR ASSESSING ALTERED DOPAMINE TRANSMISSION ............ 104
4.4.1 Pharmacological stimulation of dopamine release with d-amphetamine .......... 104
4.4.2 Pharmacological depletion of dopamine transmission with AMPT ................. 106
4.4.3 “Pathological” dopamine measurement: Parkinson disease and the dopamine system ................................................................................................................................. 108
4.5 PARTICIPANTS ........................................................................................................... 110
4.5.1 General inclusion and exclusion criteria .............................................................. 110
4.6 PET AND MRI SCANNING PROTOCOL .................................................................. 111
4.7 GENERAL DATA ANALYSIS ...............................................................................112
4.8 PARTIAL VOLUME CORRECTION ....................................................................113
4.9 STATISTICAL ANALYSIS ..................................................................................115
4.9.1 Multiple comparison control .....................................................................116

5 STUDY 1: PET IMAGING OF DOPAMINE D_{2} RECEPTORS AND EXTRACELLULAR DOPAMINE WITH [{\textsuperscript{18}}F]FALLYPRIDE, D-AMPHETAMINE, AND ALPHA-METHYL-PARA-TYROSINE IN HEALTHY SUBJECTS: EXPLORATION WITH COGNITION .............................................................................117
5.1 INTRODUCTION ..............................................................................................117
5.2 METHODS .........................................................................................................119
5.2.1 Study population .........................................................................................119
5.2.2 Radiopharmaceutical preparation ...............................................................120
5.2.3 Scanning protocol .......................................................................................120
5.2.4 Neuropsychological tests ...........................................................................123
5.2.5 Data analysis ...............................................................................................126
5.2.6 Statistical analysis ......................................................................................128
5.3 RESULTS ........................................................................................................128
5.3.1 [{\textsuperscript{18}}F]Fallypride uptake and \( BP_{SD} \) ..........................................128
5.3.2 Test-retest variability ..................................................................................129
5.3.3 Amphetamine-induced changes ..................................................................130
5.3.4 AMPT-induced changes ............................................................................132
5.3.5 Correlation between amphetamine and AMPT-induced changes ............133
5.4 DISCUSSION ..................................................................................................133

6 STUDY 2: PRE- AND POST-SYNAPTIC DOPAMINE IMAGING AND ITS RELATION WITH FRONTOSTRIATAL COGNITIVE FUNCTION IN PARKINSON DISEASE: PET STUDIES WITH [{\textsuperscript{11}}C]NNC 112 AND [{\textsuperscript{18}}F]FDOPA ..................................................138
6.1 INTRODUCTION ..............................................................................................138
6.2 MATERIALS AND METHODS ..........................................................................140
6.2.1 Subjects ......................................................................................................140
6.2.2 Radiopharmaceutical preparation and dosimetry ......................................141
6.2.3 Scanning protocol ......................................................................................142
6.2.4 Neuropsychological tests ...........................................................................144
6.2.5 Statistical analysis .....................................................................................145
6.2.6 Image analysis ............................................................................................145
6.3 RESULTS ........................................................................................................147
6.3.1 Demographic and neuropsychological data ..............................................147
6.3.2 [{\textsuperscript{18}}F]FDOPA uptake .........................................................................148
6.3.3 [{\textsuperscript{18}}F]FDOPA uptake in extrastriatal regions ................................148
6.3.4 Partial volume correction of [{\textsuperscript{18}}F]FDOPA PET .............................149
6.3.5 [{\textsuperscript{11}}C]NNC 112 binding ....................................................................150
6.3.6 Correlational analyses .................................................................151
6.4 DISCUSSION .....................................................................................152
   6.4.1 Presynaptic dopamine synthesis and questionable measurement of cortical
         \[^{18}\text{F}]\text{FDOPA} .................................................................153
   6.4.2 Postsynaptic dopamine D\textsubscript{1} receptors in Parkinson disease ........155
   6.4.3 Frontostriatal cognitive function in Parkinson disease and association with pre-
         and postsynaptic dopamine markers ..........................................156
6.5 CONCLUSION ..................................................................................158

7 GENERAL DISCUSSION AND CONCLUSIONS .....................................159
   7.1 OVERVIEW AND SUMMARY OF FINDINGS ...................................159
   7.2 GENERAL DISCUSSION AND IMPLICATIONS .................................162
      7.2.1 Striatal and extrastriatal assessment of dopamine function with PET ....162
      7.2.2 Discussion of “positive” striatal and extrastriatal imaging findings ....165
      7.2.3 Discussion of “negative” striatal and extrastriatal imaging findings ....167
      7.2.4 Quantification issues: error in the quantification of cortical \[^{18}\text{F}]\text{FDOPA} ....175
      7.2.5 Using molecular imaging as a means to explore the neurochemical
            underpinnings of human cognitive function ................................177
      7.2.6 Limitations of the current experimental studies ............................183
      7.2.7 Relevance and implications of the current findings ......................185
      7.2.8 Future research directions ........................................................187
      7.2.9 Concluding remarks ...............................................................191

8 REFERENCES .....................................................................................193

9 APPENDICES ..................................................................................251

APPENDIX 1: Reprint of Cropley et al 2006a (Biological Psychiatry)
APPENDIX 2: Reprint of Cropley et al 2008 (Synapse)
APPENDIX 3: Reprint of Cropley et al (in press) (uncorrected proof in Psychiatry Research:
               Neuroimaging)
APPENDIX 4: Reprint of Cropley et al 2006b (Journal of Nuclear Medicine)
APPENDIX 5: Poster presented at “Dopamine 50 Years” symposium in Göteborg, Sweden
               (May 2007)
Thesis overview

In May 2007, the University of Göteborg, Sweden, hosted ‘Dopamine 50 Years’, an international symposium to mark the 50th anniversary of the discovery of dopamine in the nervous system and the subsequent 50 years of dopamine research (see Bjorklund and Dunnett 2007b; Iversen and Iversen 2007). As demonstrated at this symposium, the development of sophisticated tools and novel approaches for assessing the dopamine system has been largely responsible for the numerous advances within the dopamine field over the last 50 years. The advent of molecular imaging with positron emission tomography (PET) and single photon emission computed tomography (SPECT) is arguably one of the most notable developments of the last 30 years for examination of brain dopamine function in humans. By use of radiolabeled compounds for dopamine receptors, the dopamine transporter or substrates for biosynthetic enzymes, this technique has provided new ways to assess the functional state of the dopamine system, in vivo, in living human brain. Until recently, molecular imaging of the dopamine system has been constrained to only the striatum, thus limiting its wider application in many neuropsychiatric disorders and behavioural functions that involve extrastriatal dopaminergic mechanisms. Recently however, radioligands suitable for measuring dopamine targets in extrastriatal regions have been developed, affording the possibility to examine the different components of the dopamine system not only in the striatum, but also in extrastriatal regions in normal and diseased brain.

Therefore, the general objective of this thesis was to assess, by means of two independent experimental PET studies in human volunteers, the feasibility of imaging pre-, post- and intra-synaptic components of the dopamine system in both striatal and extrastriatal regions.

This thesis contains seven chapters. Chapter 1 provides an introduction to the human dopamine system, followed by a review of molecular imaging as a research technique to assess the human dopamine system. The molecular imaging section is divided into two main components. First, following a brief description of other research methods
available for examination of brain dopamine, the chapter introduces the methodology of PET and SPECT and outlines several key areas of significant and recent advancement. The latter component of this chapter reviews molecular imaging studies of selective components of the dopamine system by use of radioligands, particularly focusing on human disorders characterised by dopamine abnormalities. Chapter 2 reviews dopamine’s involvement in cognitive function and introduces a recent application of molecular imaging techniques to investigate the role of dopamine in human cognitive processes. Chapter 3 describes the rationale and general aims and research questions of this thesis, and Chapter 4 addresses theoretical principles and general methodology relating to this thesis. Two independent, experimental studies are presented in chapters 5 and 6. Finally, Chapter 7 presents the conclusions and implications of this thesis, as well as limitations and directions for future research.
Chapter One

1 Introduction: Molecular imaging of the dopamine system

1.1 THE DOPAMINE SYSTEM

1.1.1 Introduction and history

Dopamine is the most recently discovered catecholamine neurotransmitter in the mammalian brain, discovered only 50 years ago by Nobel Laureate, Arvid Carlsson and colleagues in Lund, Sweden (Carlsson et al 1957; Carlsson et al 1958) and Kathleen Montagu in London (Montagu 1957). Previously, dopamine was exclusively considered to be an intermediary in the synthesis of the catecholamines norepinephrine and epinephrine from tyrosine. In 1957–1958, Carlsson and co-workers observed that the dopamine precursor L-3,4-dihydroxyphenylalanine (L-dopa) but not the serotonin (5-HT) precursor 5-hydroxytryptophan, reversed the akinetic effects of reserpine-treated rabbits, which was associated with the accumulation of dopamine, but not norepinephrine, content in the brain (Carlsson et al 1957; Carlsson et al 1958). At the same time, Montagu (1957) also demonstrated the presence of dopamine in animal brain. Subsequent findings showing that the majority of dopamine was concentrated in the basal ganglia (Bertler and Rosengren 1959; Sano et al 1959) and that the regional distribution of dopamine was markedly different to that of norepinephrine (Carlsson et al 1962; Dahlstrom and Fuxe 1964), suggested a distinct biological role for dopamine as a neurotransmitter independent of its role as a precursor to norepinephrine. Early research of this catecholamine transmitter focused on the putative involvement of dopamine in Parkinson disease (PD) and schizophrenia, and its role in motor function. Ehringer and Hornykiewicz (1960) were the first to demonstrate profound depletion of dopamine in the striatum of PD patients, which lead to the first trial with L-dopa for its treatment (Birkmayer and Hornykiewicz 1961) although it took several more years for a clinically efficacious L-dopa dosing treatment to be established (Cotzias et al 1967). At a similar time, various findings linking the mode of action of neuroleptic drugs to dopamine receptor antagonism, and observations that anti-psychotics could block amphetamine-induced behavioural stereotypies in the rat were central to the emergence
of the classical “dopamine hypothesis” of schizophrenia, relating over-activity of dopamine transmission to the pathophysiology of the illness (see Iversen and Iversen 2007). These seminal findings, along with advances in the tools to examine the dopamine system and the characterization of the dopamine receptors, were likely a driving force behind the dramatic increase in dopamine research over the last five decades, such that more than 100,000 articles relating to dopamine are now listed in PubMed. Dopamine is not only implicated in motor function and clinical disorders such as PD and schizophrenia, but in a range of behaviours including cognition (see Chapter 2), attention, motivation, reward prediction and addiction (Grace et al 2007; Nieoullon 2002; Schultz 2007; Volkow et al 2007). The following section presents a brief overview of the dopamine system as related to dopamine pathways in the brain, dopamine synthesis and release, its inactivation and metabolism, followed by dopamine receptors and modes of regulating dopamine transmission.

1.1.2 Dopaminergic pathways

Dopaminergic neurons are classified into 3 major classes depending on the length of the dopaminergic axons. The long dopaminergic projections consist of 3 separate tracts: 1) nigrostriatal, 2) mesolimbic, and 3) mesocortical projections (see Figure 1-1). The nigrostriatal system originates in the neuronal cell group A9, which largely corresponds to the substantia nigra pars compacta, and primarily projects to the nucleus caudate and putamen (striatum). The nigrostriatal pathway is severely damaged in PD (see Chapter 4 for further detail) and is critically involved in the regulation of movement. The mesolimbic pathway predominately originates in neuronal cell group A10, which corresponds to the ventral tegmental area, and innervates mesial components of the limbic system including the nucleus accumbens, olfactory tubercle, the lateral septal nuclei, amygdaloid complex and piriform cortex. The A10 cell group (ventral tegmental area) is also the prominent source of the mesocortical pathway which primarily innervates cortical areas including the PFC, the septum, amygdala and hippocampus (Cooper et al 2003; Meyer and Quenzer 2005). In addition to these long dopaminergic ascending projections, there are local ultrashort and intermediate length dopaminergic pathways such as the tuberoinfundibular pathway (involved in the regulation of
prolactin secretion by the pituitary gland) and the incertohypothalamic pathway (Cooper et al 2003), but these will not be discussed further in this thesis.

![Image removed for copyright protection – see printed version](image_url)

**Figure 1-1.** The main dopamine projections in the human brain. Figure reproduced from Szabo et al (2000).

It should be noted that the description of anatomically distinct nigral and ventral tegmental projection systems described above (with substantia nigra neurons projecting to the striatum and ventral tegmental neurons projecting to limbic and cortical targets) is an oversimplification. For instance, striatal dopamine innervation not only originates from the substantia nigra but also the lateral ventral tegmental area and A8 (retrorubral) neurons, while the source of mesolimbic and mesocortical pathways is not restricted to the ventral tegmentum but also includes the dorsal tier of the substantia nigra and A8 cell group (Bjorklund and Dunnett 2007a). In summary, although the striatum is a major projection target for dopaminergic neurons, the cerebral cortex and limbic structures are also highly innervated by dopamine neurons, with the source of these projections including the substantia nigra, ventral tegmental area and retrorubral area.
1.1.3 Synthesis and release of dopamine

The synthesis of dopamine occurs in several biochemical steps, as is shown in Figure 1-2. The biochemical pathway originates from the neutral amino acid L-tyrosine (tyrosine), which occurs naturally from dietary protein and is transported from blood to brain. Tyrosine is converted to L-3,4-dihydroxyphenylalanine (L-dopa) by the enzyme tyrosine hydroxylase (TH) (Nagatsu et al 1964), a reaction that requires the TH cofactor 6-tetrahydrobiopterin (BH$_4$). Because of the slow rate of conversion from tyrosine to L-dopa, TH is the rate-limiting enzyme in the synthesis of dopamine, determining the overall rate of dopamine formation. Although dopamine synthesis cannot feasibly be enhanced by increasing brain levels of tyrosine, it can be reduced (along with the transmitter norepinephrine) by the drug $\alpha$-methyl-para-tyrosine (AMPT), an inhibitor of TH (Sjoerdsma et al 1965). (See Chapter 4 for further discussion of AMPT as a methodology for depleting catecholamines.) L-dopa is subsequently converted to dopamine by the enzyme aromatic L-aromatic amino acid decarboxylase (AADC). Because the rate of conversion from L-dopa to dopamine is quite rapid, endogenous L-dopa levels in the brain are normally very low, and consequently increasing the amount of L-dopa for the enzyme AADC can augment the synthesis of dopamine (Cooper et al 2003). Dopamine synthesis is primarily regulated through mechanisms that modulate TH activity. These include the availability of the TH cofactor BH$_4$, which competes with dopamine for a binding site on TH, synthesis-modulating pre-synaptic autoreceptors and the rate of impulse flow of the dopamine neuron (Cooper et al 2003). Following its synthesis, dopamine is sequestered into storage vesicles for later release by the vesicular monoamine transporter 2 (VMAT2) or is metabolised in the cytoplasm. Dopamine is normally released from the nerve terminal into the synaptic cleft in response to an action potential, via the process of calcium-dependent exocytosis (Moore and Bloom 1979). Dopamine release is primarily regulated by pre-synaptic release-modulating autoreceptors and by the rate and pattern of dopamine neuronal firing (see section below). Figure 1-2 presents a typical dopaminergic neuron, summarizing its synthesis, storage, release, re-uptake and metabolism.
Figure 1-2. Schematic diagram of a typical dopamine synapse showing the pre-synaptic dopamine neuron and post-synaptic neuron. Tyrosine (Tyr) is converted to L-dopa (DOPA) by the enzyme tyrosine hydroxylase (TH), which is subsequently converted to dopamine (DA) (black dots) by L-aromatic amino acid decarboxylase (L-AAD or AADC). Dopamine released into the synapse is rapidly taken up into the pre-synaptic terminal by the dopamine transporter (DAT) or metabolised by, for example, monoamine oxidase B, or catechol-O-methyltransferase (COMT). Synthesis and release modulating autoreceptors are D2 receptors located on the pre-synaptic neuron. Dopamine D1 receptors are coupled to Gs protein whereas dopamine D2 receptors are coupled to Gi protein. Diagram modified from Mehta and Riedel (2006).

1.1.4 Dopamine termination: reuptake and metabolism

Inactivation of dopamine occurs via the processes of reuptake and metabolism. Dopamine nerve terminals contain high-affinity dopamine-uptake sites, specifically a membrane carrier called the dopamine transporter (DAT). Reuptake of dopamine is important for terminating dopamine action and for maintaining homeostasis (Cooper et al. 2003). Much of the released dopamine is transported back to the terminal and recycled into vesicles for re-release, while the remainder is metabolised. DAT serves as a primary regulator of intra-synaptic dopamine levels, which thereby reflects the homeostatic tone of the dopaminergic system (Jaber et al 1997; Jones et al 1998). Because DATs are localised on the presynaptic dopamine terminal, they serve as markers of dopamine neurons and dopamine nerve terminals (Volkow et al 1996b). Although the highest density of DAT is found in the striatum and nucleus accumbens,
lower levels of DAT are found in the olfactory tubercle, substantia nigra, thalamus, limbic regions and medial temporal cortex (Ciliax et al 1995; Donnan et al 1991; Hall et al 1999; Little et al 1995). A DAT protein is illustrated on the presynaptic dopamine terminal in Figure 1-2.

The metabolism of dopamine can occur in the synaptic cleft, in the cytoplasm of the presynaptic nerve terminal, and in glial cells. The main enzymes involved in the metabolism of dopamine are catechol-O-methyltransferase (COMT), monoamine oxidase (MAO) and its intermediate aldehyde dehydrogenase (AD), which convert dopamine into its main metabolites, homovanillic acid (HVA), dihydroxyphenylacetic acid (DOPAC) and 3-methoxytyramine (3-MT). Released dopamine is converted by COMT to 3-MT, which is further metabolised by MAO and AD to HVA, probably at an extraneuronal site. In dopaminergic nerve terminals, dopamine is converted to DOPAC by intraneuronal MAO and AD. After diffusing out of the neuron, DOPAC can be further metabolised to HVA by COMT (Cooper et al 2003). COMT is considered to be an important regulator of released dopamine in the prefrontal cortex (PFC) (Gogos et al 1998; Karoum et al 1994), where DAT is in low abundance (Lewis et al 2001; Sesack et al 1998). Figure 1-3 shows the main pathways for the metabolism of dopamine.

![Diagram](Image removed for copyright protection – see printed version)

**Figure 1-3.** Pathways for the metabolism of dopamine in the brain. Diagram reproduced from Volkow et al (1996b).

### 1.1.5 Dopamine receptors

Dopamine receptors are present both pre- and post-synaptically. At the post-synaptic cell, dopamine receptors function in cell-cell communication and at the pre-synaptic cell
they modulate the release and synthesis of dopamine (Jackson and Westlind-Danielsson 1994). Dopamine receptors are metabotropic receptors, belonging to the family of 7 transmembrane domain G-protein coupled receptors. There are five distinct subtypes of dopamine receptors classified into D1- and D2-like subfamilies on the basis of their biochemical and pharmacological properties. The D1-like subfamily comprises D1 and D5 receptors while the D2-like subfamily includes D2, D3 and D4 receptors (Sibley and Monsma 1992; Vallone et al 2000). These subtypes were originally classified according to their effects (positive or negative) on adenylyl cyclase (AC), the enzyme that converts adenosine triphosphate (ATP) to the second-messenger substance, cyclic adenosine monophosphate (cAMP) (Cooper et al 2003; Meyer and Quenzer 2005). D1-like receptors generally stimulate AC via a G protein (Gs) to increase cAMP formation, while D2-like receptors generally inhibit AC via a Gi protein, decreasing cAMP (Kebabian and Calne 1979; Missale et al 1998; Sibley and Monsma 1992). These opposing signaling mechanisms of D1 and D2 receptors are illustrated in Figure 1-4. Dopamine D2 receptor stimulation may also decrease intracellular calcium levels, enhance potassium channel opening or potentiate calcium-evoked arachidonic acid release (Cooper et al 2003; Missale et al 1998). D1 and D2 receptors can be expressed in a high and low affinity state (Richfield et al 1989). Those configured in the high affinity state are coupled with the G-protein, whereas those in the low-affinity state are uncoupled with the G-protein.

Figure 1-4. A simplified diagram of the dopamine receptor signalling pathways in the brain. D1 receptors configured in the high-affinity state are coupled with the Gs protein which stimulates adenylyl cyclase and increases cAMP, whereas D2 receptors configured in the high-affinity state inhibit adenylyl cyclase via a Gi protein, and decreases cAMP. Diagram taken from Meyer and Quenzer (2005).
The distribution and concentration of dopamine receptors within the brain differs. The most widely expressed are the D$_1$ (50 pmol/g) and D$_2$ (20 pmol/g) receptors, with the highest concentration of both receptors in striatum – predominately on striatal output neurons projecting to the substantia nigra for D$_1$ receptors and on striatal output neurons projecting to the globus pallidus for D$_2$ receptors (Volkow et al 1996b). The density of D$_1$- and D$_2$-like receptors are much lower (0.3–4pmol/g) in extrastriatal regions (Volkow et al 1996b). In primate PFC, D$_1$-like receptors predominate and are 10–20 fold higher than D$_2$-like receptors. The laminar distribution of D$_1$- and D$_2$-like receptors differs, with D$_1$ receptors expressed more highly in layers I, II, IIIa and VI, while D$_2$ receptors are preferentially expressed in layer V (Lidow et al 1991). Amongst the receptor subtypes, in situ hybridization studies of dopamine receptor mRNAs confirm that the D$_1$ and D$_2$ subtypes are the most ubiquitous and concentrated. In addition to the striatum and cerebral cortex, D$_1$ mRNA is mainly present in the nucleus accumbens, olfactory tubercle, amygdala and thalamus. D$_5$ receptor mRNA is mainly restricted to the hippocampus, lateral mammillary nucleus and the parafascicular nucleus of the thalamus, but is also present in cerebral cortex (Missale et al 1998; Vallone et al 2000). Both pre- and post-synaptic D$_1$ and D$_5$ receptors are found in the PFC, although post-synaptic receptors are more frequent (Missale et al 1998). Dopamine itself has ~10 times higher affinity for the D$_5$ than D$_1$ subtype (Sunahara et al 1991; Tiberi et al 1991). Like D$_1$ receptors, D$_2$ mRNA is expressed predominately in the striatum, olfactory tubercle and nucleus accumbens, but is also present in cerebral cortex, amygdala, hippocampus, substantia nigra and ventral tegmental area (Missale et al 1998; Vallone et al 2000). D$_3$ receptors exist in low concentrations (1 pmol/g) in the shell of the nucleus accumbens and in the islands of Calleja (Murray et al 1992). D$_3$ mRNA is also found in the substantia nigra pars compacta and ventral tegmental area in low levels (Missale et al 1998). Dopamine has 20 times higher affinity for D$_3$ than D$_2$ receptors (Sokoloff et al 1990). D$_4$ receptors are present in low concentrations (2.1 pmol/g tissue) in several limbic and cortical areas, with low levels found in the basal ganglia (Missale et al 1998; Seeman et al 1993; Strange 1994). Dopamine autoreceptors, which are located on the dopamine neuron itself (including the soma, dendrites and nerve terminal), are primarily of the D$_2$ type (Cooper et al 2003; Grace 2002).
Although dopaminergic ligands easily discriminate between the D₁- and D₂-like receptor subfamilies most do not easily differentiate between members of the same subfamily (e.g. between D₁ and D₅ subtypes and between D₂, D₃ and D₄ subtypes). Therefore, for brevity and consistency with much of the literature, this thesis will refer to the D₁-like and D₂-like receptor subfamilies as D₁ and D₂ receptors, respectively, unless otherwise stated. As such, the selectivity of the radioligands investigated in each of the experimental chapters will generally be limited to the receptor subfamily (D₁- and D₂-like).

1.1.6 Regulation of dopamine release: phasic and tonic modes of dopamine transmission

As previously mentioned, dopamine autoreceptors are prominent regulators of dopamine neuron activity and release. Stimulation of somatodendritic autoreceptors inhibit spike firing while stimulation of nerve terminal autoreceptors inhibits presynaptic dopamine synthesis and release (Cooper et al 2003). The extent of dopamine release is also a function of the rate and pattern of firing. Dopamine neurons display three main patterns of activity: an inactive, hyperpolarized state; a slow (2−10 Hz), single spike or ‘tonic’ firing pattern; and a burst firing or ‘phasic’ activity (Grace and Bunney 1983; Grace et al 2007). The tonic and phasic firing patterns are proposed to underlie two functionally independent components of dopamine release – termed phasic and tonic release (Grace 1991). Phasic dopamine release is characterized by a high-amplitude (millimolar range), transient increase in dopamine released within or near the synapse in response to burst firing of dopamine neurons. This is the functionally relevant signal, which stimulates post-synaptic dopamine receptors before being rapidly removed by DAT. In contrast, tonic dopamine release represents the stable, baseline level of extrasynaptic dopamine which exists in low concentration (nanomolar range) and is mediated by spike or tonic firing of dopamine neurons (Grace 1991; Grace et al 2007). Tonic dopamine levels primarily stimulate extrasynaptic receptors including dopamine terminal autoreceptors, which thereby exert feedback-inhibition of phasic dopamine release. Thus, tonic dopamine release is proposed to regulate the intensity of the phasic dopamine response (Grace 1991; Grace 1993).
1.2 ASSESSMENT OF THE DOPAMINE SYSTEM: MOLECULAR IMAGING

Numerous *in vitro*, *ex vivo* and *in vivo* research techniques exist to assess the brain dopamine system. These techniques, which are continuously increasing in number and sophistication, have greatly advanced our understanding of this neurotransmitter system – from its molecular and biochemical structure and neuronal organization to its functional role in behaviour and disease. Common techniques and research methodologies include: histofluorescence (as used by Carlsson et al 1962; Dahlstrom and Fuxe 1964) and retrograde tracing, aiding in the visualization and subsequent mapping of catecholamines in the brain; immunohistochemistry techniques for catecholamine-synthesizing enzymes such as TH or AADC, which have allowed greater characterisation of the organisation of the brain catecholamine systems (see Bjorklund and Dunnett 2007a); *in vitro* or *in vivo* receptor autoradiography or radioligand binding assays for the distribution, concentration and affinity characterization of dopamine receptors; and in situ hybridization techniques for the distribution and concentration of dopamine protein mRNA (see Meyer and Quenzer 2005). Microdialysis and *in vivo* voltammetry allows measurement of extracellular dopamine release *in vivo* in intact brain of freely moving, or behaviorally-engaged animals, while various electrophysiological recording techniques, such as intracellular and extracellular single-unit recording, are useful for measuring the activity of individual dopamine neurons under various conditions, in either anesthetized or awake, mobile animals (Meyer and Quenzer 2005). Chemical lesioning of target brain regions with specific neurotoxins for catecholamine neurons, such as 6-hydroxydopamine, is useful for experimentally investigating the behavioural and clinical effects of regional catecholamine depletion. This technique is commonly used as a model of PD in the rat (Simola et al 2007), while it also proved valuable in determining the importance of PFC dopamine for cognitive (particularly spatial working memory) behavior (Brozoski et al 1979) (see Chapter 2).

Although such methods have been, and are, significant to the study of the dopamine system, particularly at a biochemical level, none of these methods can be used in living human brain. Although autoradiography techniques using radiolabeled receptor ligands, antibodies or RNA (receptor autoradiography, immunocytochemistry and in situ hybridization, respectively) can determine the distribution of dopamine receptors, enzymes and mRNA in human brain tissue, this is obtained post-mortem in autopsy.
This is often hindered by various methodological aspects such as post-mortem delay and deterioration, the effects of age, agonal state and drug therapy and differences in quantitative methods (see Rossor 1984; Zilles et al 1988).

Molecular imaging with positron emission tomography (PET) and single photon emission computed tomography (SPECT) is a nuclear medicine technique that uses short-lived radiopharmaceuticals to image the regional distribution and kinetics of chemical compounds within the living (as opposed to post-mortem) brain. Molecular imaging is the direct in vivo correlates of in vitro autoradiographic film techniques described above, and can simply be described as “in vivo molecular imaging.” (Fujita and Innis 2002). The basic premise of PET and SPECT is similar, although compounds are labelled with positron-emitting radioisotopes (such as $^{11}$C, $^{18}$F and $^{13}$N) with subsequent dual photon emission for PET, but with gamma-emitting radioisotopes (such as $^{99}$Tc and $^{123}$I) and single photon emission for SPECT. The distribution of PET and SPECT radioligands throughout the body and brain can be externally measured and quantified in the tissues of interest as a function of time. Although dopamine was initially labelled with $^{11}$C nearly 40 years ago, allowing measurement of catecholamine metabolism in peripheral organs (Christman et al 1970), dopamine cannot cross the blood brain barrier and, thus, cannot be directly measured in brain. Therefore, imaging of the dopamine system in brain is achieved through using biochemical or radiopharmaceutical markers of dopamine receptors, transporters or enzymes (as outlined in the previous section but revisit below). As such, molecular imaging is an unsurpassed method for directly measuring components of the dopamine system in living human subjects, providing information that could only previously be assessed in animals, cell cultures or in post-mortem human brain. Although still a relatively new methodology, molecular imaging has undergone substantial progress over the last decade. The following section briefly describes several areas of significant advancement that has enabled more selective assessment of pre-, intra- and post-synaptic components of the dopamine system, the examination of not only striatal but extrastriatal regions, as well as the examination of small brain regions and subdivisions and the frontostriatal circuitry. Further detail of the principles of molecular imaging technology, specifically relating to PET, can be found in Chapter 4.
1.2.1 Assessment of pre-, intra- and post-synaptic components of the dopamine system

As mentioned above, imaging of dopamine is reliant on the development of radioligands that selectively label its various components, such as dopamine receptors, transporters or enzymes important in its synthesis or metabolism. These various constituents of the dopamine system were briefly described in the preceding section, The Dopamine System. In general, the dopamine system can be compartmentalised into pre-synaptic, post-synaptic and intra-synaptic components. Pre-synaptic components include the enzymatic reactions involved in the synthesis of dopamine (TH and AADC) and the reuptake of dopamine via DAT. Post-synaptic components include post-synaptic dopamine receptors and subsequent post-synaptic signal transduction. The intra-synaptic component of the dopamine system refers to the release or level of dopamine within or near the synapse. A wide range of PET and SPECT radioligands have become available that can assess these pre-synaptic, post-synaptic, and also intra-synaptic components of the dopamine system (see Figure 1-5). Pre-synaptic markers of dopamine neurons include the biomarker for AADC, $[^{18}\text{F}]$FDOPA, and radioligands for DAT. Radioligands for the vesicular monamine transporter, such as $[^{11}\text{C}]$tetrabenazine (Kilbourn et al 1993) and $[^{11}\text{C}]$methoxytetrabenazine (Vander Borght et al 1995) are also available for imaging of vesicular storage, but as these are substrates for not only dopamine but also noradrenaline, adrenaline, serotonin and histamine these will not be discussed further. Post-synaptic components of the dopamine system are assessed with a variety of D$_1$ and D$_2$ receptor family radioligands. Although efforts have recently been directed to developing selective radioligands for the D$_3$, D$_4$ and D$_5$ subtype, these have been met with limited success in humans. For estimation of intra-synaptic components of the dopamine system, competition for receptor binding between certain D$_2$ radioligands and endogenous dopamine has been assessed (see Laruelle 2000). Table 1-1 lists common PET and SPECT radioligands targeting the dopamine system that have been used in humans (note that this list is not exhaustive). Although numerous PET and SPECT radioligands exist, the experimental chapters of this thesis will examine three PET radioligands for assessment of pre-, post- and intra-synaptic components of the dopamine system.
1.2.2 Examination of striatal and extrastriatal regions

In early years, molecular imaging studies of the dopamine system were largely limited to the striatum, in part due to its dense dopamine innervation (and target molecules like DAT and D₁ and D₂ receptors) and limitations in scanner instrumentation. Since the mid 1990’s however, the development of dopamine receptor radioligands with higher affinity and specific to non-displaceable ratios, together with improvements in scanner sensitivity, has allowed extrastriatal as well as striatal regions to be measured. Several PET D₂ antagonist radioligands, such as [¹⁸F]fallypride and [¹¹C]FLB 457, can image and quantify low density regions such as the thalamus, amygdala, medial temporal and frontal cortices (Farde et al 1997; Mukherjee et al 2005). In addition, the SPECT radioligand, [¹²³I]epidepride, can measure D₂ receptors in temporal cortex (Fujita et al 1999; Ichise et al 1999). The D₁ receptor radioligand, [¹¹C]NNC 112, provides a superior cortical signal than older D₁ ligands such as [¹¹C]SCH 23390 (Halldin et al 1998), while the pre-synaptic radioligand, [¹⁸F]FDOPA, has also reportedly been measured in extrastriatal regions such as the cortex (e.g. Nagano et al 2000). The quantification of radioligands in extrastriatal regions allows the different components (pre-, post- and intra-synaptic) of dopamine to be examined not only in the striatum, but...
also in various limbic and cortical regions. As outlined above, these regions are also the
target of ascending dopaminergic tracts (i.e. the mesolimbic and mesocortical dopamine
pathways). As extrastriatal dopamine transmission is hypothesised to be significantly
involved in cognition (see Chapter 2), addiction, reward and psychosis (Arnsten 1998;
Floresco and Magyar 2006; Laviolette and Grace 2006; Stevens 1991) and may be the
site of action for antipsychotic drugs (Lidow et al 1998), imaging of extrastriatal human
brain is important in the study of various neuropsychiatric disorders and to the study of
dopaminergic inputs in cognition (see Chapter 2). This thesis will examine pre-, post-
and intra-synaptic dopamine components in the striatum, as well as extrastriatal areas.

1.2.3 Examination of smaller brain regions and subdivisions and subsequent
examination of the frontostriatal circuitry

As mentioned above, initial dopamine imaging studies were restricted to large structures
like the striatum. In addition to radioligand characteristics, this was also due to the low
spatial resolution of the scanner and resulting partial volume effects (i.e. loss of signal)
of small structures, limiting their accurate quantification (Rousset et al 1998). See
chapter 4 for further discussion of spatial resolution and partial volume effects.
Improvements in spatial resolution, sensitivity and imaging techniques (such as the
coregistration of emission images to structural magnetic resonance imaging scans, MRI,
or correction of partial volume effects) now allow smaller brain regions to be visualized
and delineated, such as striatal subdivisions and the substantia nigra. Quantification of
smaller subregions is important, as many brain structures are functionally and
neurochemically heterogeneous. With these advances, PET studies have segregated the
striatum into several subdivisions, such as the ventral and dorsal striata, and further
subdivisions of the caudate and putamen (e.g. Mawlawi et al 2001). This delineation
and quantification of striatal subdivisions has provided a basis for examining the
frontostriatal or fronto-striato-thalamic circuits (the neuronal loops connecting the
frontal cortex, thalamus and striatum) (Alexander et al 1986), of which the
dopaminergic projections are intricately a part of. Five discrete fronto-striatao-thalamic
circuits have been proposed – the motor, dorsolateral, orbitofrontal, limbic and
oculomotor circuits (see Figure 1-6). These anatomically and functionally segregated
pathways are organised in a parallel manner, involve different sectors of the basal
ganglia, thalamus and frontal cortex, and subserve a different set of behaviours. Thus,
the “motor” loop connects precentral motor fields and putamen and is hypothesized to underlie skeletomotor processes, while the “dorsolateral” and “orbitofrontal” loops (also referred together as the “associative” or “prefrontal” circuit) connects the dorsolateral and orbitofrontal prefrontal cortices to regions of the caudate nucleus (dorsolateral and ventromedial head, respectively) and are hypothesized to be important for cognitive processes (Alexander et al 1990; Alexander et al 1986).

Figure 1-6. A schematic diagram of the fronto-striato-thalamic circuits, reproduced from Kaasinen and Rinne (2002). GPi, internal globus pallidus; SNr, substantia nigra pars reticulata.

Molecular (and functional) imaging of small structures and substructures can therefore be used to examine the functional segregation of the frontostriatal circuitry into “motor”, “cognitive” and “limbic” domains, and to examine the dopaminergic contribution to the behaviours the circuits subserve. Parkinson disease patients have, in particular, provided a useful framework, as the disease is characterised by uneven patterns of striatal dopamine depletion, which differentially affects the frontostriatal circuits (Owen 2004). Thus, the frontostriatal circuitry model is useful for examining the neural basis of motor symptoms, cognitive deficits and drug responses in PD (Kaasinen and Rinne 2002). With respect to the current thesis, this model of basal
ganglia or striatal organisation highlights the importance to not view brain structures and substructures (i.e. the striatum and cortical regions) in isolation, but part of extended neural networks or loops. These neural circuits (i.e. the “motor” and “associative” loops) are discussed in Chapter 2 and Chapter 6 of this thesis.

<table>
<thead>
<tr>
<th>Pre-synaptic binding system</th>
<th>Radioligand</th>
<th>Post-synaptic binding system</th>
<th>Radioligand</th>
</tr>
</thead>
</table>

1.2.4 Multi-tracer imaging approach to the examination of dopaminergic mechanisms

Over recent years, more studies of dopamine imaging with PET and SPECT have performed multiple scans in the same subject, using different radioligands to examine interactions between various aspects of dopamine transmission that may be altered in pathology or implicated in various behavioural processes. For instance, using markers for DAT and post-synaptic dopamine receptors, the interaction between pre- and post-synaptic dopamine transmission in selected regions can be examined. Likewise, the interaction between D1 and D2 receptors, which has been proposed to be abnormal in the cortex of schizophrenia patients (Winterer and Weinberger 2004) may be assessed. The use of multiple tracer studies may provide additional information about the dynamic
state of the dopamine system in normal processes and disease states. Study 2 of the current thesis will employ a multi-tracer approach to explore the relationship between pre- and post-synaptic dopamine function in patients with PD.

The key areas of advancement described above will be a focus of the current thesis. Specifically, this thesis will 1) examine pre-, post- and intra-synaptic components of the dopamine system, 2) will examine the feasibility of such assessment in striatal as well as extrastriatal areas, 3) will examine striatal subdivisions and address the fronto-striatao-thalamic circuits, and 4) will use a multi-tracer design to explore relations between dopamine components.

1.3 MOLECULAR IMAGING OF THE DOPAMINE SYSTEM IN THE HUMAN BRAIN: A SELECTED REVIEW

The above section introduced molecular imaging with PET and SPECT as a powerful method to visualise and study the dopamine system in human brain and described several areas that have significantly advanced within the field. This section will present a broad overview of the PET and SPECT studies that have imaged the dopamine system in human subjects, paying particular attention to the areas of advancement described above, i.e. extrastriatal and subdivision examination of novel dopamine components. The general components of the dopamine system will be covered: pre-synaptic (dopamine synthesis and reuptake); post-synaptic (D₁-like and D₂-like dopamine receptors) and intra-synaptic (phasic and tonic dopamine levels). The measurement of these components in extrastriatal regions will be addressed when applicable. Although PET and SPECT studies of dopamine radioligands are widely performed in rodents and non-human primates, especially in the initial stages of radioligand development and evaluation, this review will primarily cover human studies, due to the human focus of the current thesis. The section will restrict the review to human studies in healthy subjects (generally healthy aging) and disorders characterised by alteration of dopamine transmission (PD and schizophrenia). Although broad in scope, this review is intended to provide a background to the various radioligands used in dopamine imaging studies, their implementation in normal and patient populations and report the general findings of such studies.
1.3.1 Assessment of pre-synaptic dopamine function

Dopamine synthesis

$[^{18}\text{F}]$FDOPA

Radioligands for dopamine synthesis enable assessment of TH and AADC activity – the enzymes involved in the hydroxylation of tyrosine to L-dopa, and subsequent decarboxylation of L-dopa to dopamine. The most widely used radioligand for studies of dopamine synthesis is $6-[^{18}\text{F}]$fluoro-L-3,4-dihydroxyphenylalanine ($6-[^{18}\text{F}]$FDOPA), a fluorinated analog of L-dopa, which was first used in the human brain in the early 1980’s (Garnett et al 1983). Two other ring-fluorinated analogs of L-dopa have also been developed – 2-$[^{18}\text{F}]$FDOPA and 5-$[^{18}\text{F}]$FDOPA. Of the three fluorinated analogs, $6-[^{18}\text{F}]$FDOPA is the more potent, due to its lower affinity for COMT, and higher affinity for AADC, than its other fluorinated counterparts (Borri Voltattorni et al 2002; Creveling and Kirk 1985; Cumming et al 1988). Thus, $6-[^{18}\text{F}]$FDOPA is the more suitable radioligand for PET imaging and extensively used for assessment of pre-synaptic dopamine function.

Like L-dopa, $6-[^{18}\text{F}]$FDOPA (referred to as $[^{18}\text{F}]$FDOPA from this point on), crosses the blood brain barrier, resulting in a unidirectional accumulation of $^{18}\text{F}$ in tissue, particularly the striatum. This accumulation (or uptake) reflects the decarboxylation of fluorodopa to fluorodopamine by AADC and the subsequent storage of fluorodopamine within vesicles (Hoshi et al 1993; Volkow et al 1996b). $[^{18}\text{F}]$FDOPA uptake constants correlate with nigrostriatal neuronal density in humans (Snow et al 1993) and in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine- (MPTP) treated monkeys (Pate et al 1993), as well as with striatal activities of the synthesis enzymes (TH and AADC) and levels of dopamine in MPTP-treated monkeys (Pate et al 1993). Thus, $[^{18}\text{F}]$FDOPA PET is considered to provide a good measure of the integrity of the nigrostriatal dopamine system (Pate et al 1993; Volkow et al 1996b). Nevertheless, a disadvantage of $[^{18}\text{F}]$FDOPA concerns its labeled metabolites, particularly 3-$O$-methyl-$[^{18}\text{F}]$FDOPA (3-OMFD), which contribute to the measured PET signal (Dhawan et al 1996). $[^{18}\text{F}]$FDOPA, like L-dopa, undergoes extensive $O$-metylation to 3-OMFD by COMT, which can also penetrate the blood brain barrier, while decarboxylated $[^{18}\text{F}]$fluorodopamine is metabolised by COMT and MAO to its $^{18}\text{F}$ labeled acidic metabolites, DOPAC (FDOPAC), and HVA (FHVA) (Cumming and Gjedde 1998) (see
Figure 1-7). While inhibition of peripheral COMT activity with agents such as entacapone have been used to increase the availability (or half-life) of $[^{18}\text{F}]$FDOPA in plasma, which has increased striatal $[^{18}\text{F}]$FDOPA uptake (Ishikawa et al 1996a; Ruottinen et al 1995; Sawle et al 1994), this is not a standard procedure in $[^{18}\text{F}]$FDOPA PET imaging. Rather, the administration of peripheral inhibitors of AADC, such as carbidopa, is customary, to inhibit peripheral metabolism of $[^{18}\text{F}]$FDOPA and subsequently increase its availability in the brain (Melega et al 1990). Nevertheless, a downside to peripheral AADC inhibition is increased $O$-methylation of $[^{18}\text{F}]$FDOPA to 3-OMFD, which can then enter the brain and contribute to the measured (non-specific) radioactivity (Volkow et al 1996b). Because AADC is not only present in dopaminergic neurons but also serotonergic and noradrenergic neurons (Tison et al 1991), $[^{18}\text{F}]$FDOPA is not solely specific to pre-synaptic dopamine function. Although this should not pose a problem for striatal $[^{18}\text{F}]$FDOPA measurements because of the dense dopaminergic innervation in this region, $[^{18}\text{F}]$FDOPA measurements in extrastriatal areas (see below) is the sum of AADC activity in all three monoaminergic neurons (dopamine, norepinephrine, and serotonin).

Image removed for copyright protection – see printed version

Figure 1-7. Simplified graphical representation of the metabolism of $[^{18}\text{F}]$FDOPA in the peripheral circulation and brain. Following injection, $[^{18}\text{F}]$FDOPA crosses the blood brain barrier where it is subsequently decarboxylated to FDA by AADC. The availability of cerebral $[^{18}\text{F}]$FDOPA is reduced by peripheral decarboxylation of FDOPA as well as $O$-methylation to 3-OMFD. The radioactivity in brain detected by PET is not only contained to $^{18}\text{F}$- FDOPA and FDA, but also $^{18}\text{F}$ labelled 3-OMFD and FDA metabolites. Diagram modified from Cumming and Gjedde (1998).
[18F]FDOPA assessment in Parkinson disease

PET imaging with [18F]FDOPA is widely used to quantify loss of nigrostriatal dopamine terminal function in PD, where a marked and consistent loss of [18F]FDOPA uptake in the striatum of patients compared to controls is observed (Brooks and Piccini 2006). Over the last three decades, data has accumulated that has used [18F]FDOPA PET to examine the nigrostriatal dopamine system at different stages of PD (such as early to advanced stages), monitor disease progression and severity, detect preclinical disease states in subjects at risk for PD, differentiate PD from other neurodegenerative and parkinsonian syndrome disorders, and correlate [18F]FDOPA changes with clinical decline (including motor as well as cognitive decline) within the framework of the frontostriatal neural circuitry. For instance, in line with the uneven pathology of PD (Kish et al 1988), a characteristic pattern of [18F]FDOPA uptake reductions is seen in PD patients throughout the different stages of the disease. In early, unilateral PD (hemiparkinson), [18F]FDOPA uptake is shown to be preserved in the caudate, but reduced in the bilateral dorsal putamen, with greater reductions seen in the dorsal putamen contralateral to the clinically affected side (Morrish et al 1995). As the disease progresses to affect all limbs, loss of [18F]FDOPA uptake is extended to ventral and anterior putamen and dorsal caudate with dorsal putamen decreasing further. The ventral caudate however, is affected only later in the disease (Brooks and Piccini 2006; Morrish et al 1996b). At the time of symptom onset, [18F]FDOPA uptake is reduced by about 30% in the putamen contralateral to the affected limbs, but by about 50% in the most posterior aspect of the putamen (Brooks and Piccini 2006; Morrish et al 1995; Morrish et al 1996b). Serial [18F]FDOPA PET studies have also allowed objective monitoring of disease progression in PD, whereby the average annual rate of decline of putamen [18F]FDOPA uptake has been calculated to be 8 – 12% of the baseline mean (Morrish et al 1998; Morrish et al 1996a; Nurmi et al 2001). [18F]FDOPA measurement in PD has also been shown to correlate with clinical deterioration, which is a core criterion of a progression indicator (Brooks et al 2003). Several studies have shown a correlation between striatal (particularly putamen) [18F]FDOPA uptake reductions in PD and locomotor severity (measured by scales such as the Unified Parkinson’s Disease Rating Scale, UPDRS) (Brooks et al 1990b; Broussolle et al 1999; Morrish et al 1995; Nagano-Saito et al 2004), particularly ratings of bradykinesia and rigidity (Brooks and Piccini 2006; Otsuka et al 1996; Vingerhoets et al 1997). Furthermore, some studies have reported an inverse correlation between [18F]FDOPA uptake in the caudate and
cognitive impairment (Cropley et al 2006a) (refer to Chapter 2 for further detail). Thus these studies provide indirect support for the concept of basal ganglia organisation into distinct frontostriatal loops.

Several studies have used $^{[18F]}$FDOPA PET to detect preclinical dopaminergic dysfunction in at risk subjects for PD, such as clinically unaffected twins or relatives of patients with PD (Brooks 1991; Burn et al 1992; Laihinen et al 2000; Piccini et al 1999; Piccini et al 1997). These studies have shown a higher concordance in at-risk subjects, indicating a role of inheritance in even sporadic PD. Lastly, although it is clinically important to distinguish PD from other parkinsonian syndromes, $^{[18F]}$FDOPA has only modest discriminate validity for such differential diagnoses. Although $^{[18F]}$FDOPA has been shown to discriminate PD from essential tremor cases (Brooks et al 1992b), it cannot reliably distinguish PD from other syndromes such as multiple system atrophy and progressive supranuclear palsy (Burn et al 1994). However, multitracer imaging studies (Heiss and Hilker 2004) and examination of striatal patterns of $^{[18F]}$FDOPA uptake (Brooks et al 1990a) may aid in this process.

Measurement of $^{[18F]}$FDOPA in extrastriatal regions

Although early studies of $^{[18F]}$FDOPA uptake have been concentrated to the striatum, with advances in scanner resolution and sensitivity as well as 3D-MRI voxel-based methods of PET analysis, recent imaging studies have also examined $^{[18F]}$FDOPA uptake in extrastriatal regions such as the lateral and medial frontal cortex, midbrain and various limbic structures. For example, in normal elderly subjects, high $^{[18F]}$FDOPA uptake values were observed in the midbrain, amygdala, hippocampus and medial PFC in addition to the striatum (Nagano et al 2000). Other studies have reported differences in pre-synaptic dopamine synthesis in extrastriatal regions between PD patients and controls, implicating disruption of not only nigrostriatal but mesolimbic and mesocortical dopamine projections (but alternatively monaminergic projections) in the pathology of PD. These changes tend to be dependent on the stage of the disease. In early PD, increased $^{[18F]}$FDOPA uptake has been reported in prefrontal and medial frontal cortex (including the anterior cingulate), amygdala and midbrain (Bruck et al 2005; Kaasinen et al 2001; Rakshi et al 1999), although reduced $^{[18F]}$FDOPA uptake has also been observed in the midbrain (substantia nigra) in one study (Ito et al 1999b). In contrast, as the disease progresses to moderate to advanced stages, $^{[18F]}$FDOPA
uptake has been shown to either decrease or normalise in various extrastriatal regions in addition to the striatum. Decreases have been observed in midbrain (Ito et al 2002; Rakshi et al 1999) and frontal cortex (Rinne et al 2000) in PD patients compared to controls, while further reductions have been observed in anterior cingulate in PD patients with dementia (Ito et al 2002). In contrast to early PD, Rakshi and co-workers (1999) reported no change in $[^{18}\text{F}]$FDOPA uptake in anterior cingulate and amygdala in advanced PD, indicating subsequent normalisation of dopamine synthesis in these regions. Therefore, the increases in prefrontal and medial frontal cortex in early PD may reflect compensatory upregulation of dopamine synthesis in intact mesocorticolicombic dopamine pathways, which subsequently degenerate (along with serotonergic and noradrenergic neurons) as the disease advances. The $[^{18}\text{F}]$FDOPA signal, particularly in midbrain, is likely a complex summation of dopaminergic, serotonergic and noradrenergic systems, and may reflect neuronal cell loss as well as adaptive upregulation mechanisms (Heiss and Hilker 2004). This apparent capability of PET to visualise and quantify extrastriatal dopamine synthesis with $[^{18}\text{F}]$FDOPA is exciting as it allows the mesolimbic and mesocortical dopamine pathways to be examined. The contribution of extrastriatal dopamine synthesis to the cognitive deficits seen in PD is reviewed in Chapter 2 of this thesis. Nevertheless, although these results are exciting, the reliability and specificity of the cortical $[^{18}\text{F}]$FDOPA signal to dopamine synthesis is still uncertain, due to low specific-to-nonspecific ratios outside the striatum.

$[^{18}\text{F}]$FDOPA assessment in other groups (healthy aging and schizophrenia)

Studies examining the effect of age on nigrostriatal dopamine function with $[^{18}\text{F}]$FDOPA are inconsistent. Some studies have observed an age-related decline in $[^{18}\text{F}]$FDOPA uptake or dopamine storage capacity (Cordes et al 1994; Kumakura et al 2005; Martin et al 1989), while others have found no age-associated change (Eidelberg et al 1993; Ishikawa et al 1996b; Sawle et al 1990). While differences in study samples, design and data analysis may have contributed to these varying results, $[^{18}\text{F}]$FDOPA PET may be an insensitive indicator of small age-related change in dopamine nerve terminals and function. This is supported by a post-mortem and PET study suggesting that AADC is upregulated in dopamine neurons that are spared during aging, and therefore $[^{18}\text{F}]$FDOPA measurements may overestimate the number of nerve terminals during normal aging (Kish et al 1995).
Further, pre-synaptic dopamine synthesis using $[^{18}\text{F}]$FDOPA has been assessed in medicated and unmedicated patients with schizophrenia. Consistent with the dopamine overactivity hypothesis of schizophrenia, elevated dopamine synthesis, indicated by increased $[^{18}\text{F}]$FDOPA uptake, has been observed in the striatum of unmedicated (Hietala et al 1999; Hietala et al 1995; Meyer-Lindenberg et al 2002; Reith et al 1994) and medicated (McGowan et al 2004b) patients. Nevertheless, no difference in $[^{18}\text{F}]$FDOPA uptake was detected in one study (DaoCastellana et al 1997), whereas reduced striatal $[^{18}\text{F}]$FDOPA uptake was observed in another (Elkashef et al 2000). In addition to accelerated striatal dopamine synthesis, a recent $[^{18}\text{F}]$FDOPA PET study has reported elevated $[^{18}\text{F}]$fluorodopamine turnover in the striatum, midbrain and amygdala of unmedicated schizophrenia patients, indicating a primary abnormality in the vesicular storage or retention of labelled dopamine in untreated schizophrenia brain (Kumakura et al 2007). These in vivo $[^{18}\text{F}]$FDOPA PET studies suggest abnormal pre-synaptic striatal dopamine function in schizophrenia, reflected by not only increased capacity for dopamine synthesis, but increased dopamine turnover and functional dysregulation of dopamine catabolism.

Other markers for pre-synaptic dopamine synthesis

Several other radioligands have been developed to measure pre-synaptic dopamine synthesis, although none are as widely used as $[^{18}\text{F}]$FDOPA. L-dopa labelled in the β-position with carbon 11 (L-$[^{11}\text{C}]$DOPA) was developed in 1990 by Bjurling and colleagues (Bjurling et al 1990) and has been used to assess pre-synaptic dopamine function in schizophrenia (Gefvert et al 2003; Lindstrom et al 1999) and PD (Tedroff et al 1996a). While the shorter half-life of $^{11}\text{C}$ (20.3 minutes) provides the benefit of performing multiple scans in the same individual, the complicated radiosynthesis of L-$[^{11}\text{C}]$DOPA has caused it to be used in only a limited number of PET centres (Elsinga et al 2006). Other tracers for dopamine synthesis include $[^{18}\text{F}]$fluoro-m-tyrosine ($[^{18}\text{F}]$FMT) (Melega et al 1989), which is a substrate for AADC but not for COMT (Volkow et al 1996b) and $[^{18}\text{F}]$fluoro-β-fluoro-methylene-m-tyrosine (DeJesus et al 1992). Like $[^{18}\text{F}]$FDOPA, $[^{18}\text{F}]$FMT is an effective tracer for examining AADC activity as an index of pre-synaptic dopamine integrity, however, it is not as effective as $[^{18}\text{F}]$FDOPA for determining in vivo estimates of dopamine turnover (Doudet et al 1999).
**Dopamine transporter (DAT)**

Pre-synaptic dopamine function is also commonly assessed *in vivo* with radioligands that target DAT, the protein involved in reuptake of synaptically released dopamine. A variety of PET and SPECT probes for DAT have been developed (see Table 1-1), which differ in respect to their affinity and specificity for DAT, signal-to-noise ratios, kinetic profiles and suitability for clinical use. Because the experimental chapters of this thesis will not evaluate radioligands for DAT, this section will provide only a brief overview of the human DAT imaging literature. The first DAT radioligand developed for PET was $[^{11}C]$nomifensine (Aquilonius et al 1987; Salmon et al 1990), but it has similar affinity for both the DAT and norepinephrine transporter (Volkow et al 1996b). Other early DAT radioligands include $[^{18}F]$GBR 13119 (Kilbourn 1988; Kilbourn et al 1989), $[^{11}C]$cocaine (Fowler et al 1989), $[^{11}C]d$-threo-methylphenidate (Ding et al 1994), and the cocaine analogs, $[^{123}I]β$-CIT (also known as RTI-55) (Innis et al 1993; Neumeyer et al 1991) and $[^{11}C]β$-CFT (also known as WIN 35428) (Madras et al 1989; Wong et al 1993). $β$-CFT, which shows peak striatal binding at 225 min post injection – more than 10 half-lives of $^{11}C$, can also be labeled with $^{18}F$ ($[^{18}F]β$-CFT) to allow PET scanning to be conducted at binding equilibrium (Haaparanta et al 1996; Laakso et al 1998). $[^{11}C]$Cocaine is limited due to its relatively low signal-to-noise ratio (< 2) and rapid uptake and clearance from the brain (maximum striatal uptake occurs 4–7 min post injection and half-life is ~25 min) (Shih et al 2006; Telang et al 1999), whereas $[^{11}C]d$-threo-methylphenidate shows slower kinetics than $[^{11}C]$cocaine, thus making it more suitable for quantification (Volkow et al 1995; Volkow et al 1996b). The SPECT tracer $[^{123}I]β$-CIT is probably the most commonly used DAT radioligand, at least in the clinical assessment of PD. $[^{123}I]β$-CIT exhibits very high affinity for the DAT and high specific-to-nonspecific binding (Innis et al 1993), although a disadvantage of this ligand is its low selectivity towards DAT, where it binds with high affinity to both DAT and the serotonin transporter (Neumeyer et al 1991). Nevertheless, the vast majority of striatal $[^{123}I]β$-CIT activity is proposed to be associated with DAT (Laruelle et al 1993). More recent DAT radioligands include $[^{123}I]/[^{18}F]$FP-CIT (Abi-Dargham et al 1996; Chaly et al 1996), $[^{99mTc}]TRODAT-1$ (Kung et al 1996), $[^{123}I]/[^{11}C]$altropane (Fischman et al 2001; Madras et al 1998), $[^{11}C]/[^{123}I]$PE2I (Guilloteau et al 1998) and $[^{18}F]$FECNT (Goodman et al 2000). An advantage of $[^{99mTc}]TRODAT-1$ is its availability and easy preparation with a kit formulation (Choi et al 1999), thus making it
accessible for routine clinical use (Shih et al 2006). Although the recent compounds $[^{11}\text{C}]$PE2I and $[^{18}\text{F}]$FECNT show good selectivity for DAT and favourable brain kinetics (Davis et al 2003; Halldin et al 2003), they both generate radiometabolites that may confound brain radioligand measurements (Shetty et al 2007; Zoghbi et al 2006). Although *in vivo* measurement of extrastriatal DAT binding is limited, mainly due to the low DAT density outside the striatum, several studies have successfully measured DAT binding *in vivo* in human thalamus, limbic and paralimbic structures and midbrain (Leroy et al 2007; Ouchi et al 1999b; Telang et al 1999).

Clinically, PET and SPECT imaging of DAT radioligand binding is sometimes used to assess dopamine terminal denervation in PD. Because DAT tracers simply measure the density of the DAT protein, DAT imaging is proposed to be a more sensitive indicator of dopamine neuronal loss than $[^{18}\text{F}]$FDOPA (Fujita and Innis 2002), which may undergo compensatory upregulation mechanisms and thus underestimate the level of dopamine denervation (see $[^{18}\text{F}]$FDOPA section above). An additional benefit of DAT imaging over $[^{18}\text{F}]$FDOPA PET imaging is that DAT ligands can be quantified with SPECT, which is less costly and more accessible for clinical practice than PET. Loss of DAT has been shown to parallel loss of dopamine in the striatum of MPTP-treated monkeys (Bezard et al 2001). Like $[^{18}\text{F}]$FDOPA, DAT radioligand binding can clearly discriminate established and clinical probable PD from normal controls and also from essential tremor cases with greater than 90% specificity (Asenbaum et al 1998; Brooks and Piccini 2006; Lee et al 1999; Poewe and Scherfler 2003). Likewise, DAT imaging is capable of detecting presymptomatic nigrostriatal dysfunction in at-risk subjects (Brooks and Piccini 2006; Poewe and Scherfler 2003), such as elderly subjects with hyposmia (diminished sense of smell) (Ponsen et al 2004). Loss of striatal DAT binding significantly correlates with degree of motor disability in PD which used over time can monitor disease progression and severity (Brooks et al 2003; Poewe and Scherfler 2003), and may be useful to monitor the efficacy of putative neuroprotective treatments (Fujita and Innis 2002; Poewe and Scherfler 2003). As with $[^{18}\text{F}]$FDOPA imaging, DAT imaging cannot differentiate PD from other related parkinsonian disorders (Poewe and Scherfler 2003).

In contrast to $[^{18}\text{F}]$FDOPA, but consistent with post-mortem data (De Keyser et al 1990b), DAT radioligand binding decreases with normal aging by about 3 – 8% per
decade (Fischman et al 1998; Ishikawa et al 1996b; Mozley et al 1999; van Dyck et al 2002; van Dyck et al 1995; Volkow et al 1996a; Volkow et al 1994a). This age-related decline in DAT availability appears to be non-linear, with faster rates of decline seen in younger than older adults (Mozley et al 1999). Furthermore, age-related decline of striatal DAT may mediate age-related cognitive deficits (Erixon-Lindroth et al 2005) (see Chapter 2). Assessment of striatal DAT binding in schizophrenia using various PET and SPECT probes has been relatively inconsistent. In general, first-episode, neuroleptic-naïve schizophrenic patients have shown no difference in striatal DAT availability compared to healthy controls (Hsiao et al 2003; Laakso et al 2000; Lavalaye et al 2001; Schmitt et al 2006; Schmitt et al 2005; Yang et al 2004b), although a decrease in binding has been reported in one study (Mateos et al 2007). The density of striatal DAT in medicated schizophrenic patients however, is contradictory, with decreases (Laakso et al 2001; Mateos et al 2005; Mateos et al 2007), increases (Sjoholm et al 2004) and no change (Laruelle et al 2000; Lavalaye et al 2001) in DAT availability being reported. Clearly, differences in DAT radioligand and the characteristics of the patient population, including duration and type of treatment, illness phase, age, and severity of symptoms, will add to the variability and make comparisons difficult. Although no overall difference in dopamine terminal density or DAT expression appears to occur in neuroleptic- naïve schizophrenia, these individuals may show a lack of asymmetry in striatal DAT binding (Hsiao et al 2003; Laakso et al 2000) or a functional change in the interaction between DAT and D₂ receptors using a dual-tracer imaging approach (Schmitt et al 2007; Yang et al 2004b). Recent studies have also characterised subgroups of schizophrenic patients based on the extent of psychopathology (Schmitt et al 2006; Schmitt et al 2007), and have observed higher DAT binding in patients with predominantly positive symptoms (Schmitt et al 2007), and associations between DAT and core symptoms of psychosis (Schmitt et al 2006; Schmitt et al 2007). As DAT is a primary target for the effects of several psychostimulant drugs, such as cocaine, methylphenidate and amphetamine (Amara and Kuhar 1993; Giros and Caron 1993), assessment of DAT radioligand binding in drug addiction and in the reinforcing properties of drugs has been of interest. For a review of DAT imaging in drug reinforcement and addiction readers are referred to Volkow et al (1999a; 2004).
1.3.2 Assessment of post-synaptic dopamine receptors

**Dopamine D<sub>2</sub> receptor measurement**

Post-synaptic dopamine function is commonly investigated via assessment of D<sub>2</sub> receptors. A variety of D<sub>2</sub> receptor agonists and antagonists have been labelled for use with PET and SPECT (see Table 1-1). These radioligands differ with respect to their affinity and specificity for the D<sub>2</sub> receptor, signal-to-noise ratios and kinetic properties. Early D<sub>2</sub> receptor antagonist radioligands include butyrophenones [<sup>11</sup>C]N-methylspiperone (Wagner et al 1983), [<sup>18</sup>F]-N-methylspiroperdol (Arnett et al 1986), and [<sup>18</sup>F]-fluoroethyl-spiperone (Coenen et al 1987), and the benzamides [<sup>11</sup>C]raclopride (Ehrin et al 1985; Farde 1986) and [<sup>123</sup>I]IBZM (Kung et al 1989). [<sup>11</sup>C]N-methylspiperone ([<sup>11</sup>C]NMSP), which was the main radioligand used during the 1980’s, has a relatively high affinity for the D<sub>2</sub> receptor (K<sub>D</sub> = 0.05 – 0.3 nM, *in vitro*) but also binds to the 5-HT<sub>2</sub>, D<sub>3</sub> and D<sub>4</sub> receptor (Lyon et al 1986; Seeman et al 1993). [<sup>11</sup>C]Raclopride, on the other hand, has moderate affinity (K<sub>D</sub> = 1 - 2 nM, *in vitro*) (Seeman et al 1989) but a greater selectivity for D<sub>2</sub> receptors, although, like other benzamide derivatives it also binds to D<sub>3</sub> receptors (Halldin et al 2001). Because [<sup>11</sup>C]raclopride has a modest affinity and specific-to-nonspecific binding ratios, accurate quantification is restricted to high D<sub>2</sub> density regions such as the striatum. Striatal [<sup>11</sup>C]raclopride binding in healthy subjects shows high test-retest reliability, both short and long-term (Schlosser et al 1998; Volkow et al 1993) The most common SPECT radioligand for assessment of striatal D<sub>2</sub>-like receptors is [<sup>123</sup>I]IBZM (K<sub>D</sub> = 0.4 nM, *in vitro*) (Kung et al 1988). While this ligand has properties (i.e. long half-life and slow washout) suitable for studying pharmacological modulation of the dopamine system, it also has a relatively poor target-to-background signal (Seibyl et al 1992). An alternative SPECT D<sub>2</sub> radioligand is [<sup>123</sup>I]IBF (Kung et al 1990).

As mentioned previously, the development of radioligands with higher affinity for the D<sub>2</sub> receptor and higher specific-to-nonspecific ratios has permitted accurate visualisation and quantification of D<sub>2</sub> receptors in extrastriatal regions. Several high affinity ligands have been developed for this purpose, such as the benzamide derivatives [<sup>11</sup>C]FLB 457 (K<sub>D</sub> = 0.02 nM, *in vitro*) (Halldin et al 1995), [<sup>123</sup>I]epidepride (K<sub>D</sub> = 0.03 nM, *in vitro*) (Kessler et al 1991) and [<sup>18</sup>F]fallypride (K<sub>D</sub> = 0.03 nM, *in vitro*) (Mukherjee et al 1995). These radioligands show high accumulation of radioactivity in
the striatum and several low density D₂ receptor regions, and show high ratios of target-to-background activity in human subjects (Kornhuber et al 1995; Kuikka et al 1997; Mukherjee et al 2002; Olsson et al 1999). As mentioned above, these radioligands have successfully quantified D₂ receptors in several extrastriatal regions including the thalamus, amygdala and cerebral cortex (Farde et al 1997; Fujita et al 1999; Mukherjee et al 2005). Nevertheless, the in vivo kinetics of high-affinity tracers has been shown to be slower in high density regions (Laruelle et al 1994), which makes it problematic for quantification in these areas. Although [¹¹C]FLB 457 shows transient-equilibrium of binding in several brain regions such as the cerebral cortex within 60 min of injection, radioactivity in the striatum is still increasing at this time and transient-equilibrium is not reached in the striatum within the time frame of the PET measurement (Farde et al 1997; Olsson et al 1999). Likewise, using an equilibrium paradigm to account for the presence of lipophilic metabolites, [¹²³I]epidepride requires approximately 24 h constant infusion for the tracer to achieve equilibrium receptor binding levels in the striatum, of which a trade-off for such a long scanning protocol is poor counting statistics in low-density extrastriatal regions (Fujita et al 1999). Because of this long time to reach binding equilibrium in high-density regions, [¹¹C]FLB 457 and [¹²³I]epidepride are generally useful for quantification of D₂ receptors in extrastriatal regions only (Fujita et al 2000; Okubo et al 1999). In contrast to [¹¹C]FLB 457 and [¹²³I]epidepride however, a plateau in the striatal time-activity curve of [¹⁸F]fallypride can be seen at approximately 2 hours after tracer injection with a decline thereafter, which, with the longer half-life of [¹⁸F], enables quantification of D₂ receptors in the striatum with a prolonged imaging time-frame of approximately 2 - 3 hours (Christian et al 2000). Like [¹¹C]raclopride, [¹¹C]FLB 457, [¹²³I]epidepride and [¹⁸F]fallypride are not D₂ receptor selective, but bind to both D₂ and D₃ receptors. Although there is a need to develop pure selective dopamine receptor radioligands for all the D₂ receptor family subtypes, i.e. D₂, D₃ and D₄ receptors, there are, at present, no suitable PET or SPECT radioligands for the subtypes (see Elsinga et al 2006). Nevertheless, because the ratio of D₂/D₃ receptors is high in most brain regions (see previous section), the PET and SPECT measurements from these ligands, particularly in the striatum, predominately reflect binding to D₂ receptors.

In recent years, a major focus of PET and SPECT radioligand development has been the labelling of D₂ receptor agonists. These agonist radioligands include the apomorphine
analogs $[^{11}C]$NPA (Hwang et al 2000) and $[^{11}C]$MNPA (Finnema et al 2005), and $[^{11}C]$PHNO (Wilson et al 2005), which has recently been shown to preferentially label the D<sub>3</sub> receptor in vivo (Narendran et al 2006). Agonist ligands are advantageous as they can provide more information about the high-affinity D<sub>2</sub> receptor sites and are more sensitive to changes in endogenous dopamine levels. The use of agonist radioligands to probe intra-synaptic dopamine neurotransmission will be discussed in section 1.4.3.

D<sub>2</sub> receptor measurement in schizophrenia

Because D<sub>2</sub> receptors are the main therapeutic target in the treatment of psychotic symptoms in schizophrenia (Seeman and Van Tol 1994), examination of D<sub>2</sub> receptors in the schizophrenic brain has been of primary interest. As discussed in several reviews (Frankle and Laruelle 2002; Kegeles and Mann 1997), studies of D<sub>2</sub> receptor measurement in schizophrenia, particularly in early studies, has been inconsistent. For instance, using $[^{11}C]$NMSP, Wong et al (1986) reported elevated D<sub>2</sub> receptor density in the striatum of medication-free, medication-naïve and medication-withdrawn schizophrenia patients compared to healthy controls. In contrast, Farde et al (1990; 1987b) failed to replicate these findings with $[^{11}C]$raclopride, where no group differences in D<sub>2</sub> receptor levels were reported. Subsequent studies to these reports using different D<sub>2</sub> radioligands have also produced variable results. In addition to differences in study population, these discrepant results have been attributed to differences in the characteristics of the radioligands used. Seeman et al (1989) was the first to propose that raclopride is more vulnerable to competition by endogenous dopamine than spiperone, and thus, differences in synaptic dopamine concentration between schizophrenia patients and controls could obscure D<sub>2</sub> receptor measurement due to differences in availability of receptor binding sites for the two ligands. While this characteristic alone does not fully explain the discrepant findings, this hypothesis paved the way for applying competitive binding techniques to assess synaptic dopamine concentration in vivo (Laruelle 2000) (see below). Differences between butyrophenones (like spiperone) and benzamides (like raclopride) for binding to D<sub>4</sub> receptors was also hypothesised to account for the discrepant results of the aforementioned studies. Seeman et al (1993) demonstrated a 6-fold elevation of D<sub>4</sub> receptors in the post-mortem striatum of schizophrenic patients, which may be detected by only $[^{11}C]$NMSP, as $[^{11}C]$NMSP, but not $[^{11}C]$raclopride, labels the D<sub>4</sub> receptor subtype. Nevertheless, some doubt has been cast on this explanation from subsequent studies (see Kegeles and Mann...
Meta-analyses of D$_2$ receptor binding studies in schizophrenia (including post-mortem and in vivo imaging studies) (Kestler et al. 2001; Weinberger and Laruelle 2002), have demonstrated a small, but significant, elevation of striatal D$_2$ receptors and a larger variability of D$_2$ receptor indices in patients with schizophrenia compared to healthy controls. These effects are amplified in medicated schizophrenia patients, and tend to be larger for post-mortem than in vivo imaging studies (Kestler et al. 2001). Further, in vivo studies performed with butyrophenone radioligands were shown to have a significantly larger effect size than studies performed with other radioligands including benzamides, suggesting an increase in butyrophenone, but not benzamide, binding in schizophrenia (Weinberger and Laruelle 2002). Therefore, based on these meta-analyses, patients with schizophrenia show a modest elevation of striatal D$_2$ receptors which are better detected by butyrophenone than benzamide radioligands, and, although are better seen in post-mortem (of likely older and medicated) schizophrenia brain, are also observed in vivo. Further, the greater variability of D$_2$ receptors observed in schizophrenia suggests heterogeneity of the disorder which, along with underpowered studies, has likely contributed to the inconsistent D$_2$ receptor imaging results in the striatum of schizophrenia patients. Although the clinical significance of increased D$_2$ receptor binding is uncertain (Weinberger and Laruelle 2002), preliminary results suggest that elevated striatal D$_2$ receptor binding with $[^{123}]$IBZM may predict poor prognosis in neuroleptic-naïve schizophrenia patients (Perez et al. 2003).

With the use of extrastriatal D$_2$ radioligands, D$_2$ receptors have recently been measured in extrastriatal regions of schizophrenia. Several studies using $[^{11}]$CFLB 457 and $[^{18}]$Ffallypride have demonstrated reduced D$_2$ receptors in the thalamus (especially medial thalamus) of drug-naïve schizophrenia patients compared to controls (Buchsbaum et al. 2006; Talvik et al. 2003; Yasuno et al. 2004), implicating thalamic dopamine abnormalities in the illness. Decreased D$_2$ receptor availability in schizophrenia has also been observed in the cingulate cortex, amygdala, temporal cortices and midbrain (Buchsbaum et al. 2006; Suhara et al. 2002; Tuppurainen et al. 2003; Tuppurainen et al. 2006). While many studies have failed to detect striatal abnormalities of D$_2$ receptors in schizophrenia, these studies suggest that alteration of extrastriatal D$_2$ receptors may be involved in the disorder, and emphasise the
importance of developing radioligands and imaging techniques for assessment of extrastriatal dopamine transmission.

Because antipsychotics act by blockade of D$_2$ receptors, intense research has centred on assessing the relationship between in vivo receptor occupancy by antipsychotic medication and therapeutic efficacy. Receptor occupancy studies have measured level of D$_2$ (as well as D$_1$ and 5-HT$_2$) occupancy in striatal and more recently, extrastriatal regions by various typical and atypical antipsychotics, related occupancy to optimal clinical response and experience of extrapyramidal side effects, compared degree of receptor occupancy in clinical responders and non-responders, and have determined optimal dose ranges for new potential antipsychotic drugs. Review of such in vivo receptor occupancy studies are beyond the scope of the present thesis, however, please refer to Frankle and Laruelle (2002), Kapur (1998) and Pani et al (2007) for further detail.

D$_2$ receptor measurement in Parkinson disease

Studies in PD have indicated that while there may be initial increases of striatal D$_2$ receptors in the early stage of the disease, these receptors downregulate as the disease progresses to return to normal or slightly subnormal levels (Antonini et al 1997; Aquilonius 1991; Rinne et al 1990c). Although on the most part, D$_2$ receptors do not differ between normal control and PD subjects, D$_2$ receptor binding with [$^{123}$I]IBZM has been capable of predicting responsiveness to dopamine therapy in “de novo” PD patients (Hertel et al 1997; Schwarz et al 1993), at clinical patient follow-up of about 36 months (Schwarz et al 1998), and in patients with questionable or negative prior treatment response (Schwarz et al 1997). As such D$_2$ receptor imaging may identify PD patients who will benefit from dopaminergic treatment. Whether D$_2$ imaging can predict the development of motor complications is uncertain. Schwarz and colleagues (1998) demonstrated a significant correlation between [$^{123}$I]IBZM binding and the development of motor fluctuations, however Turjanski et al (1997) found no differences in either D$_1$ or D$_2$ receptor binding between dyskinetic and non-dyskinetic PD patients using L-dopa, matched for duration of clinical disease. On the other hand, Hwang et al (2002) demonstrated that downregulation of striatal D$_2$ receptors in advanced PD contributed to the development of motor fluctuations. Although pre-synaptic imaging of dopamine function with [$^{18}$F]FDOPA and DAT cannot discriminate idiopathic PD from other
Parkinsonian syndromes, *in vivo* D<sub>2</sub> imaging may aid in the differential diagnosis of PD. Reduced striatal D<sub>2</sub> binding was observed in idiopathic PD patients with a fluctuating response to L-dopa and, to a lesser extent, in patients with multiple system atrophy and progressive supranuclear palsy (Brooks et al 1992a). SPECT studies have also replicated this D<sub>2</sub> receptor reduction in multiple system atrophy and progressive supranuclear palsy but not in idiopathic PD (see Verhoeff 1999), which is probably a reflection of striatal medium spiny neuron degeneration in the former patients. Nevertheless, while D<sub>2</sub> receptor imaging for differential diagnosis is promising, the discrimination is not reliable enough for routine clinical use (Stoessl and Ruth 1998). Recently, the diagnostic value of asymmetric striatal D<sub>2</sub> receptor upregulation (contralateral to the most clinically affected side) in early PD was assessed, and although higher binding was observed in the contralateral striatum, it had insufficient diagnostic accuracy for PD (Verstappen et al 2007). To date, reduced extrastriatal D<sub>2</sub> receptors as indexed with [¹¹C]FLB 457 has been reported in advanced but not early (unmedicated) PD compared to normal controls (Kaasinen et al 2000a), while a 6 – 11% annual decline of cortical D<sub>2</sub> receptors has been demonstrated in early PD patients examined longitudinally, about 3 years apart (Kaasinen et al 2003). This rate of decline is substantially faster than that seen in normal individuals (~1%/year) (see below). However, as patients were never medicated (de novo) at the time of their first examination, but had received medication (for an average of 2.4 y) prior to their second examination, it is unknown to what extent the treatment or the disease process contributed to the D<sub>2</sub> receptor loss. In summary, while striatal D<sub>2</sub> receptors may undergo compensatory upregulation in the early stages of PD, for the most part, striatal D<sub>2</sub> receptors do not differ much between patients and age-matched controls. In contrast, preliminary studies suggest that extrastriatal D<sub>2</sub> receptors may decrease with progression of PD, although the relative effects of treatment from the disease itself cannot be distinguished.

**D<sub>2</sub> receptor measurement in healthy aging**

In line with DAT, PET and SPECT studies have consistently shown striatal D<sub>2</sub> receptor reductions with normal aging, with a greater rate of decline seen before the age of 30 – 40 years (Antonini and Leenders 1993; Volkow et al 1996c). Striatal D<sub>2</sub> receptors are reported to decline by 4 – 13% per decade using different radioligands such as [¹¹C]raclopride (Antonini and Leenders 1993; Pohjalainen et al 1998; Volkow et al
1996c), \([^{11}C]NMSP\) (Wong et al 1997), \([^{18}F]NMSP\) (Volkow et al 1996c) and \([^{123}I]IBF\) (Ichise et al 1998), with reductions in D\(_2\) binding potential (see Chapter 4 for further detail) related to decreases in receptor density but not in affinity of the D\(_2\) receptor (Rinne et al 1993). Recently, asymmetry in D\(_2\) receptors of the caudate nucleus only has been reported to be lost with age (Vernaleken et al 2007b). Age-related loss of extrastriatal D\(_2\) receptors has also been demonstrated, with declines per decade of 10 – 14\% in the frontal and temporal cortex, 13\% in the anterior cingulate and parietal cortex, 10 – 12 \% in the hippocampus, 7\% in the amygdala, and 5 – 6\% in the medial and lateral thalamus (Inoue et al 2001; Kaasinen et al 2000b; Talvik et al 2003). Talvik et al (2003) demonstrated a negative age effect on D\(_2\) receptor binding in the frontal and temporal cortex but not in the thalamus or anterior cingulate, indicating that the D\(_2\) receptor age effect differs between brain regions. Age-associated loss of D\(_2\) receptors has been associated with impairment of motor (Volkow et al 1998a) and cognitive (see Chapter 2) processes observed with normal aging.

**Dopamine D\(_1\) receptor measurement**

In contrast to in vivo imaging of D\(_2\) receptors, there are a limited number of suitable antagonist radioligands and no suitable agonist radioligands for in vivo imaging of postsynaptic D\(_1\) receptors (Elsinga et al 2006) (see Table 1-1). The first selective D\(_1\) ligand labelled with positron emitters was the benzazepine antagonist \([^{11}C]SCH 23390\) (Halldin et al 1986), followed by the benzonaphthazepine, \([^{11}C]SCH 39166\) (Halldin et al 1991), and benzazepines, \([^{11}C]NNC 687, [^{11}C]NNC 756\) (Karlsson et al 1993) and \([^{11}C]NNC 112\) (Halldin et al 1998). A disadvantage of these radioligands is that they also display affinity for 5HT\(_2\) receptors (Andersen et al 1992; Karlsson et al 1993), although for \([^{11}C]SCH 23390\) and \([^{11}C]NNC 112\), the affinity for 5HT\(_2\) is reported to be at least 100-fold lower than for D\(_1\) receptors (Halldin et al 1998) (but please refer to Chapter 7 for recent findings). Further, radioligands belonging to the benzazepine class bind to both D\(_1\) and D\(_5\) receptors (Halldin et al 2001), demonstrating a need to develop pure radioligands for the subtypes of the D\(_1\) receptor family (i.e. D\(_1\) and D\(_5\)). Although candidate D\(_1\) compounds with higher D\(_1\)/D\(_5\) selectivity have recently been reported these have not been labeled for PET, and, at present, few candidates have been described for the D\(_5\) receptor (Elsinga et al 2006). Therefore, and as mentioned
previously, D₁/D₅ (D₁-like) receptor radioligands will generally be referred to as D₁ radioligands throughout this thesis.

The most common D₁ radioligands are [¹¹C]SCH 23390 and [¹¹C]NNC 112. Both radioligands show high affinity for the D₁ receptor, with in vitro affinities of 0.14 nM or 0.4 nM for [¹¹C]SCH 23390 (Andersen et al 1992; Chipkin et al 1988) and 0.18 nM for [¹¹C]NNC 112 (Andersen et al 1992). These radioligands show high uptake in the striatum which is the region with the greatest density of D₁ receptors. [¹¹C]NNC 112, however, has higher specific to nonspecific binding ratios than [¹¹C]SCH 23390 (Haldin et al 1998), making it superior to [¹¹C]SCH 23390 for assessment of extrastriatal regions such as the neocortex where the density of D₁ receptors are low. D₁ receptor binding has been measured in extrastriatal regions such as the frontal, temporal and parietal cortices, limbic and paralimbic structures and thalamus, and have been in agreement with the known distribution of D₁ receptors in brain (Abi-Dargham et al 2000a; Abi-Dargham et al 2002). Moreover, [¹¹C]NNC 112 has shown good reproducibility of D₁ receptor parameters in striatal and extrastriatal regions (Abi-Dargham et al 2000a). Likewise, however, good test-retest reliability has been determined for [¹¹C]SCH 23390 parameters in both striatum and cortex (Chan et al 1998; Hirvonen et al 2001).

D₁ receptor measurement in healthy aging
Although post-mortem studies examining age effects on D₁ receptor density have been inconsistent, with both decreases (De Keyser et al 1990a; Rinne et al 1990b; Seeman et al 1987a) or no change (De Keyser et al 1990b; Rinne 1987) being reported, in vivo PET imaging studies suggest that D₁ receptors likely decrease with age. Using [¹¹C]SCH 23390, D₁ receptor binding has shown to decline with age in normal brain, with reductions per decade of about 6.9% in the putamen, 7.4% in the caudate, 7.5% in the frontal cortex and 8.6% in the occipital cortex (Suhara et al 1991; Wang et al 1998). Age-associated reduction of D₁ receptors may play a role in the motor decline seen in the elderly (Wang et al 1998).

D₁ receptor measurement in schizophrenia
Measurement of D₁ receptors in schizophrenia using [¹¹C]SCH 23390 or [¹¹C]NNC 112 has demonstrated no change in D₁ binding in the striatum of drug-naïve or drug-free
schizophrenia patients compared to controls (Abi-Dargham et al 2002; Karlsson et al 2002; Okubo et al 1997), although one study has reported D_1 receptor reductions in the high-intensity regions (patch component) of the basal ganglia in drug-naïve patients in comparison to controls (Sedvall et al 1995). This lack of striatal D_1 receptor alteration in schizophrenia is in agreement with the majority of post-mortem studies failing to find any changes in the densities of D_1 receptors in the basal ganglia of schizophrenic brain (Cross et al 1981; Joyce et al 1988; Knable et al 1994; Mamelak et al 1993; Pimoule et al 1985). In the prefrontal cortex however, in vivo binding of D_1 receptors in drug-naïve or drug-free schizophrenia patients is controversial, with decreases (Okubo et al 1997), increases (Abi-Dargham et al 2002) or no change (Karlsson et al 2002) in D_1 receptors being reported. Although two studies included drug-free schizophrenia patients (Abi-Dargham et al 2002; Okubo et al 1997) prior exposure to antipsychotic medication was found to have no effect on the results of D_1 receptor availability. The first and second of these studies also demonstrated a significant correlation between cognitive task performance measures and PFC receptor densities (see Chapter 2). The main difference between these three PET studies is the choice of radioligand; the studies showing reduced or no change in D_1 binding used the radioligand [11C]SCH 23390 (Karlsson et al 2002; Okubo et al 1997), while the study showing increased D_1 binding used the radioligand [11C]NNC 112.

These contrasting studies, particularly those showing opposite findings (decreased and increased D_1 binding) are difficult to reconcile. The a priori hypothesis of the investigators of these studies presumably was that patients would have decreased D_1 receptor levels, based on the dopamine hypothesis of schizophrenia which postulates that positive symptoms are associated with increased “dopamine function” in subcortical regions, while cognitive impairment is caused by decreased “dopamine function” in prefrontal cortex (Davis et al 1991; Weinberger 1987). As is common with most dopamine radioligand studies however, the definition of “dopamine function” is so vague that it can imply even contradictory changes in individual components of the dopamine system. For instance, Okubo et al (1997) claim that their finding of decreased D_1 receptor binding is consistent with overall decreased “dopamine function” in prefrontal cortex. With apparent equal face validity, Abi-Dargham et al (2002) postulate that the increased D_1 receptor binding represents a compensatory upregulation of D_1 receptors due to sustained cortical dopamine deficiency. Thus, both authors claim that
the observed alterations of D₁ receptor density reflect a common underlying deficit in prefrontal dopamine. Because of the relative paucity of studies in this area, neither of these two interpretations can be discounted.

The reasons for these contradictory results are unknown but worthy of further investigation, especially in light of the robustness of studies of prefrontal dopamine depletion and D₁ receptor function in nonhuman primates (see Chapter 2). The two patient populations seem fairly similar, at least within our current ability to distinguish subgroups of patients with schizophrenia. Differences in the specific region studied (both studies using [¹¹C]SCH 23390 sampled the prefrontal cortex as a whole while the [¹¹C]NNC 112 study segregated the prefrontal cortex into the DLPFC, orbitofrontal and medial prefrontal cortex subregions) and biochemical heterogeneity within the prefrontal cortex may also account for some of the discrepant findings. Consideration of the in vivo behaviour of the two radioligands under conditions of dopamine depletion however may offer a more likely explanation (Guo et al 2003). Following acute dopamine depletion in rodents, the in vivo binding of [¹¹C]NNC 112 was unaffected while [³H]SCH 23390 was decreased – a paradoxical response proposed to be related to dopamine depletion-induced internalization of D₁ receptors. In contrast, subchronic dopamine depletion resulted in increased binding of [¹¹C]NNC 112, which was proposed to reflect compensatory upregulation of D₁ receptors, while [³H]SCH 23390 showed either decreased or unchanged binding (Guo et al 2003). Although these studies were carried out in rodents, the finding that the two most common D₁ radioligands SCH 23390 and NNC 112 are differentially affected by dopamine depletion may be relevant to the interpretation of the above PET studies performed in schizophrenia (Abi-Dargham and Moore 2003). Studies with both radiotracers on the same schizophrenia patients are required to clarify this issue.

D₁ receptor measurement in Parkinson disease

PET studies of D₁ receptor binding in PD have all used the radioligand, [¹¹C]SCH 23390. These studies have all reported striatal D₁ receptor density to be unchanged in idiopathic, non-demented PD compared to normal controls (Ouchi et al 1999a; Shinotoh et al 1993; Turjanski et al 1997). This lack of D₁ alteration has been demonstrated in early, de novo PD patients (Ouchi et al 1999a; Shinotoh et al 1993) and moderate to advanced, medicated (but medication-free at time of scan) patients (Shinotoh et al 1993;
Further, in early, hemiparkinson (unilateral) patients, binding of $[^{11}C]SCH$ 23390 was symmetric between striatal hemispheres (Rinne et al 1990a), indicating a lack of D$_1$ receptor supersensitivity in the striatum of early PD, which is in contrast to striatal D$_2$ receptors in early PD (see D2 section above). Although Turjanski et al (1997) did not show a significant difference in caudate and putamen D$_1$ receptors between medicated PD patients compared to controls, there was a trend for reduced binding (~10%) in patients, and D$_1$ receptor binding in the putamen of PD patients was negatively correlated with duration of L-dopa treatment. Striatal D$_1$ binding was not different between PD patients who did versus did not develop dyskinesias on L-dopa (Turjanski et al 1997), indicating that D$_1$ receptors do not provide a basis for the development of motor complications in PD. To date, only one study has examined D$_1$ receptor density in vivo in cortex of PD patients, which demonstrated no change of D$_1$ receptors in the orbitofrontal cortex of early, de novo PD patients (Ouchi et al 1999a) but this study again used $[^{11}C]SCH$ 23390, which is not the radioligand of choice for measurement of extrastriatal D$_1$ receptors (see above). Using a dual-tracer imaging approach to examine pre- and post-synaptic function, Ouchi et al (1999a) also observed an asymmetrical association between DAT and D$_1$ receptors in the striatum of early PD, presumably due to a loss of pre-synaptic DAT and relative elevation of post-synaptic D$_1$. In contrast, DAT and D$_1$ receptors were observed to change in parallel in the striatum of normal subjects, which is consistent with that seen between pre-synaptic DAT and post-synaptic D$_2$ receptors with normal aging (Volkow et al 1998b).

### 1.3.3 Assessment of intra-synaptic dopamine release

**Competition between endogenous dopamine and D$_2$ radioligands: The Occupancy Model**

A novel application of in vivo neuroreceptor imaging with PET and SPECT is to measure fluctuations in synaptic concentration of neurochemicals in the brain. This approach is based on the principle of competition between the endogenous neurotransmitter (such as dopamine) and radioligand for binding to the receptor (such as D$_2$ receptors). That is, changes in the concentration of the endogenous neurotransmitter correspond to changes in receptor occupancy and availability of the receptor for radioligand binding (Laruelle 2000). Since the 1990’s, this technique has been applied...
in humans to estimate synaptic dopamine transmission via assessment of D2 receptor availability with radioligands like \(^{11}C\)raclopride and \(^{123}I\)IBZM. On the basis of the competition principle, increasing synaptic dopamine concentration will decrease D2 radioligand binding, and vice versa, thereby providing an indirect method to measure synaptic levels of dopamine. This ability to assess extracellular dopamine levels \textit{in vivo} in living human brain is exciting because it purports to isolate and examine an additional component of the dopamine system (dopamine release) which could previously only be assessed with microdialysis or \textit{in vivo} voltammetry techniques in animals.

Imaging of synaptic dopamine release according to \textit{in vivo} binding competition principles can be described within a theoretical framework called the occupancy model (Laruelle 2000) (Figure 1-8). The occupancy model predicts that changes in D2 radioligand binding (e.g. with \(^{11}C\)raclopride) are a direct result of changes in the occupancy (and hence availability) of D2 receptors by dopamine. Thus, pharmacological challenges that increase synaptic dopamine equates to higher D2 receptor occupancy by dopamine which consequently lowers the availability of D2 receptors for binding by the radioligand, whereas the reverse is true for challenges that reduce dopamine levels. In imaging studies, the validity of the occupancy model can be evaluated by comparison of radioligand binding following acute manipulation of the dopamine system to control conditions. In general, elevation of dopamine concentration initiated by stimulants such as amphetamine or methylphenidate induces a decrease in D2 radiotracer binding, while depletion of dopamine by agents such as AMPT is reflected by an increase in D2 radiotracer binding, both in comparison to baseline binding (Laruelle 2000). These stimulant-induced decreases and depletion-induced increases of radioligand binding have been proposed to loosely measure phasic and tonic dopamine release, respectively. Stimulants like amphetamine cause a rapid, high elevation of extracellular dopamine characteristic of phasic dopamine release, whereas dopamine depletion results in a low, steady-state level of dopamine representative of the tonic mode, with the percentage of “unmasking” of D2 receptors available for radioligand binding reflecting the level of D2 receptor occupancy by dopamine under ‘tonic’ or baseline conditions (see Figure 1-8) (Fujita and Innis 2002).
**Figure 1-8.** Simplified diagram of the classic ‘occupancy model’ used to explain the increases and decreases in benzamide (such as $[^{11}\text{C}]$raclopride) binding following depletion and stimulation, respectively, of synaptic dopamine. The occupancy model proposes that the increases and decreases of $D_2$ radioligand binding are due to decreased and increased, respectively, occupancy of $D_2$ receptors by dopamine. Figure reproduced from Laruelle (2000).

*In vivo* imaging of synaptic dopamine transmission has been widely performed in rodents, non-human primates and humans. A variety of $D_2$ and $D_1$ receptor radioligands have been evaluated for their vulnerability to endogenous dopamine competition to that predicted by the occupancy model, with various outcomes. A critical review of these studies, including those in rodents and non-human primates, and of those which do and do not validate the classic occupancy model, is found elsewhere (Laruelle 2000) and will not be covered here. However, in brief, the use of this technique to characterize synaptic dopamine transmission has generally been successful for benzamide $D_2$ radioligands, but not for butyrophenone $D_2$ radioligands or $D_1$ radioligands. While numerous studies have demonstrated that benzamide radioligands (such as $[^{11}\text{C}]$raclopride) behave in a fashion consistent with the occupancy model, butyrophenones (such as $[^{11}\text{C}]$NMSP) and $D_1$ receptor radioligands have shown either no change or paradoxical changes to acute dopamine manipulations (see Laruelle 2000). As mentioned in the $D_2$ imaging section above, this different vulnerability of benzamide and butyrophenone ligands may have contributed to the discrepant $D_2$ receptor findings using $[^{11}\text{C}]$raclopride and $[^{11}\text{C}]$NMSP in schizophrenia. Due to this chemical class
differentiation, imaging of dopamine transmission in humans have all used D₂ radioligands belonging to the benzamide class.

Imaging of intra-synaptic dopamine transmission in healthy humans

Although an effect of dopamine competition on [¹¹C]raclopride binding was first observed by Farde et al (1992) within a larger study of D₂ receptor occupancy with antipsychotics, the first comprehensive study that imaged intra-synaptic dopamine release in humans was not until 1994 by Volkow and colleagues (Volkow et al 1994b), who reported a 23% displacement of striatal [¹¹C]raclopride binding after intravenous methylenidate administration. This effect was shortly replicated by Laruelle and colleagues (1995) using intravenous amphetamine and [¹²³I]IBZM SPECT, who observed a 15% reduction in striatal binding. Both studies also observed correlations between the magnitude of striatal dopamine release and subjective emotional state, either with baseline (pre-drug) levels of mood and anxiety (Volkow et al 1994b) or with stimulant-induced (post-drug) levels of euphoria, alertness and restlessness (Laruelle et al 1995), suggesting a method for exploring human functional correlates of regional dopamine release. Since these initial studies, the feasibility of this approach to examine stimulant-induced (phasic) dopamine release in healthy humans has been demonstrated, with all but one study (Koochesfahani et al 2006) observing significant reductions of D₂ radioligand binding following stimulant challenges (see Table 1-2). Most studies have been restricted to the striatum, sampling the whole striatum or basic putamen and caudate divisions with [¹¹C]raclopride or [¹²³I]IBZM. Striatal displacement of radioligand binding has ranged from about 8 – 25% (see Table 1-2), indicating inter-study variability in the magnitude of stimulant-induced striatal dopamine release which is likely due to differences in radioligand, challenge, dose and route of stimulant administration, sampled region and characteristics of the study population. For the most part, [¹¹C]raclopride appears to be more vulnerable to endogenous dopamine competition, with an average striatal binding decrement of about 16% compared to about 10% using [¹²³I]IBZM. Good within-subject reproducibility of amphetamine-induced decrease in striatal [¹²³I]IBZM binding has also been established (Kegeles et al 1999), indicating an absence of either sensitisation or tolerance to the effects of amphetamine. Likewise, methylphenidate-induced decrements of striatal [¹¹C]raclopride binding is reproducible (Wang et al 1999), although is less stable than amphetamine-induced release. Despite there being stable measurements of stimulant-induced change
in synaptic dopamine over time, there is marked variability in the magnitude of radioligand displacement between-subjects. Such between-subject variability may partly be due to variances in subject age, given that the magnitude of both amphetamine- and methylphenidate-induced radioligand displacement decreases as a function of age (Volkow et al 1994b; Volkow et al 2001; Wang et al 1999), which is in line with the age-associated decline of other dopamine parameters such as DAT and dopamine receptors (see sections above). From inspection of Table 1-2, phasic dopamine release has typically been initiated via intravenous administration of psychostimulants, due to its robust effect on radioligand binding in non-human primate and human studies (see Laruelle 2000). More recent studies however, have evaluated the feasibility for oral as opposed to intravenous administration of stimulants to inhibit radioligand binding, and have demonstrated that, at least for amphetamine, oral doses are equally potent at displacing $^{11}$C]raclopride binding in the striatum as intravenous amphetamine administration (Boileau et al 2006; Boileau et al 2007; Cardenas et al 2004; Leyton et al 2002), which has beneficial ramifications for studies of this nature due to the lower risk associated with the oral versus intravenous route of stimulant administration.

Corresponding to the general advancements in molecular imaging technology previously discussed in this chapter, recent studies have attempted to evaluate regional differences in amphetamine-induced dopamine release across striatal subdivisions as well as extrastriatal regions, and have further attempted to explore the relationship between regional dopamine release and human emotion, reinforcement and other (such as cognitive) behaviours. Several studies have demonstrated differential amphetamine-induced dopamine release within the functional subdivisions of the human striatum, where the greatest release (indexed by greatest $^{11}$C]raclopride displacement) is seen in the ventral striatum (corresponding to the mesolimbic circuit) and postcommissural/ventral putamen (corresponding to the sensorimotor circuit) (Drevets et al 2001; Martinez et al 2003). Recent studies using the same radioligand ($^{11}$C]raclopride), amphetamine dose (0.3 mg/kg) but different route of administration (oral) have also demonstrated that the ventral striatum is more sensitive to the effects of amphetamine than other striatal subdivisions (Boileau et al 2006; Boileau et al 2007), a finding supported by several microdialysis studies in rats showing greater increases in extracellular dopamine in the accumbens during amphetamine challenge (Di Chiara and Imperato 1988; Sharp et al 1987). Further, recent studies indicate that the emotional (i.e.
euphoria and “drug-wanting”) response to amphetamine appears to be related to the magnitude of dopamine release in the ventral (Drevets et al 2001; Leyton et al 2002; Martinez et al 2003) and sensorimotor (Martinez et al 2003) striatum, demonstrating the capability of this in vivo imaging approach to explore the neurochemical circuits underlying human emotional responses to various drugs of abuse.

An exciting, yet preliminary development in recent years has been the assessment of stimulant-induced dopamine release in extrastriatal regions using high-affinity $D_2$ radioligands (Montgomery et al 2007; Riccardi et al 2006). These studies observed significant, but modest, stimulant-induced dopamine release in several extrastriatal regions such as the substantia nigra, amygdala, temporal cortex and thalamus using $[^{18}F]$fallypride and oral amphetamine (Riccardi et al 2006), and in the frontal and temporal cortex and thalamus using $[^{11}C]$FLB 457 and oral methylphenidate (Montgomery et al 2007). Although preliminary and of modest effect (radioligand displacement in extrastriatal areas ranged between 3 and 7%), these studies suggest that high-affinity $D_2$ radioligands are vulnerable to endogenous dopamine competition and demonstrate the feasibility for this in vivo binding competition technique to assess dopamine concentration in areas outside the striatum, which has important implications to the study of extrastriatal dopamine in neuropsychiatric disease and human behaviours.
<table>
<thead>
<tr>
<th>Study</th>
<th>Radioligand</th>
<th>Challenge</th>
<th>Dose and route</th>
<th>Effect on in vivo binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farde et al (1992)</td>
<td>[^{11}\text{C}]raclopride</td>
<td>Amphetamine</td>
<td>30 mg, oral</td>
<td>Decreased by 10% in striatum</td>
</tr>
<tr>
<td>Volkow et al (1994b)</td>
<td>[^{11}\text{C}]raclopride</td>
<td>Methylphenidate</td>
<td>0.5 mg/kg, i.v.</td>
<td>Decreased by 23% in striatum</td>
</tr>
<tr>
<td>Laruelle et al (1995)</td>
<td>[^{123}\text{I}]IBZM</td>
<td>Amphetamine</td>
<td>0.3 mg/kg, i.v.</td>
<td>Decreased by 15% in striatum</td>
</tr>
<tr>
<td>Laruelle et al (1996)</td>
<td>[^{123}\text{I}]IBZM</td>
<td>Amphetamine</td>
<td>0.3 mg/kg, i.v.</td>
<td>Decreased by 7.6% in striatum</td>
</tr>
<tr>
<td>Booij et al (1997)</td>
<td>[^{123}\text{I}]IBZM</td>
<td>Methylphenidate</td>
<td>0.5 mg/kg, i.v.</td>
<td>Decreased by 8.6% in striatum</td>
</tr>
<tr>
<td>Breier et al (1997)</td>
<td>[^{11}\text{C}]raclopride</td>
<td>Amphetamine</td>
<td>0.2 mg/kg, i.v.</td>
<td>Decreased by 15.5% in striatum</td>
</tr>
<tr>
<td>Volkow et al (1997)</td>
<td>[^{11}\text{C}]raclopride</td>
<td>Methylphenidate</td>
<td>0.5 mg/kg, i.v.</td>
<td>Decreased by 21% in striatum</td>
</tr>
<tr>
<td>Abi-Dargham et al (1998)</td>
<td>[^{123}\text{I}]IBZM</td>
<td>Amphetamine</td>
<td>0.3 mg/kg, i.v.</td>
<td>Decreased by 7% in striatum</td>
</tr>
<tr>
<td>Kegeles et al (1999)</td>
<td>[^{123}\text{I}]IBZM</td>
<td>Amphetamine</td>
<td>0.3 mg/kg, i.v.</td>
<td>Decreased by 8.7% in striatum</td>
</tr>
<tr>
<td>Volkow et al (1999b)</td>
<td>[^{11}\text{C}]raclopride</td>
<td>Methylphenidate</td>
<td>0.25 and 0.5 mg/kg, i.v.</td>
<td>Decreased by ~ 14 and 19% in striatum, respectively</td>
</tr>
<tr>
<td>Wang et al (1999)</td>
<td>[^{11}\text{C}]raclopride</td>
<td>Methylphenidate</td>
<td>0.5 mg/kg, i.v.</td>
<td>Decreased by 16% in striatum</td>
</tr>
<tr>
<td>Anand et al (2000)</td>
<td>[^{123}\text{I}]IBZM</td>
<td>Amphetamine</td>
<td>0.3 mg/kg, i.v.</td>
<td>Decreased by 14% in striatum</td>
</tr>
<tr>
<td>Drevets et al (2001)</td>
<td>[^{11}\text{C}]raclopride</td>
<td>Amphetamine</td>
<td>0.3 mg/kg, i.v.</td>
<td>Decreased by 10.6% in whole striatum; decreased by 5−15% in striatal subdivisions</td>
</tr>
<tr>
<td>Parsey et al (2001)</td>
<td>[^{123}\text{I}]IBZM</td>
<td>Amphetamine</td>
<td>0.3 mg/kg, i.v.</td>
<td>Decreased by 7.8% in striatum</td>
</tr>
<tr>
<td>Volkow et al (2001)</td>
<td>[^{11}\text{C}]raclopride</td>
<td>Methylphenidate</td>
<td>60 mg, oral</td>
<td>Decreased by 20% in striatum</td>
</tr>
</tbody>
</table>
### Table 1-2. Continued...

<table>
<thead>
<tr>
<th>Study</th>
<th>Radioligand</th>
<th>Challenge</th>
<th>Dose and route</th>
<th>Effect on binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leyton et al (2002)</td>
<td>$^{[11]}$Craclopride</td>
<td>Amphetamine</td>
<td>0.3 mg/kg, oral</td>
<td>Decreased by 10.7% in ventral striatum</td>
</tr>
<tr>
<td>Martinez et al (2003)</td>
<td>$^{[11]}$Craclopride</td>
<td>Amphetamine</td>
<td>0.3 mg/kg, i.v.</td>
<td>Decreased by 10.3% in whole striatum; decreased by 8−16% in striatal subdivisions</td>
</tr>
<tr>
<td>Piccini et al (2003)</td>
<td>$^{[11]}$Craclopride</td>
<td>Methamphetamine</td>
<td>0.3 mg/kg, i.v.</td>
<td>Decreased by 25.2% in putamen</td>
</tr>
<tr>
<td>Abi-Dargham et al (2004)</td>
<td>$^{[123]}$IBZM</td>
<td>Amphetamine</td>
<td>0.3 mg/kg, i.v.</td>
<td>Decreased by 7% in striatum</td>
</tr>
<tr>
<td>Cardenas et al (2004)</td>
<td>$^{[11]}$Craclopride</td>
<td>Amphetamine</td>
<td>30 mg, oral</td>
<td>Decreased by 13% in striatum</td>
</tr>
<tr>
<td>Boileau et al (2006)</td>
<td>$^{[11]}$Craclopride</td>
<td>Amphetamine</td>
<td>0.3 mg/kg, oral</td>
<td>Decreased by 17.7% in ventral striatum and 7.3% in postcomissural dorsal putamen</td>
</tr>
<tr>
<td>Koochesfahani et al (2006)</td>
<td>$^{[11]}$Craclopride</td>
<td>Methylphenidate</td>
<td>0.8 mg/kg, oral</td>
<td>No change</td>
</tr>
<tr>
<td>Riccardi et al (2006)</td>
<td>$^{[18]}$Fallypride</td>
<td>Amphetamine</td>
<td>0.43 mg/kg, oral</td>
<td>Decreased by 6−11% in striatal subdivisions, 7% in SN, 3-4% in amygdala, temporal ctx, thalamus</td>
</tr>
<tr>
<td>Boileau et al (2007)</td>
<td>$^{[11]}$Craclopride</td>
<td>Amphetamine</td>
<td>0.3 mg/kg, oral</td>
<td>Decreased by 22% in ventral striatum and 11% in the putamen</td>
</tr>
<tr>
<td>Montgomery et al (2007)</td>
<td>$^{[11]}$CFLB 457</td>
<td>Methylphenidate</td>
<td>40 or 60 mg, oral</td>
<td>Decreased by 5.7 – 7.2% in frontal and temporal cortex and thalamus</td>
</tr>
</tbody>
</table>

**Acute dopamine depletion: ‘Tonic Release’**

<table>
<thead>
<tr>
<th>Study</th>
<th>Radioligand</th>
<th>Challenge</th>
<th>Dose and route</th>
<th>Effect on binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laruelle et al (1997a)</td>
<td>$^{[123]}$IBZM</td>
<td>AMPT</td>
<td>8 g over 48 h, oral</td>
<td>Increased by 28% in striatum</td>
</tr>
<tr>
<td>Abi-Dargham et al (2000b)</td>
<td>$^{[123]}$IBZM</td>
<td>AMPT</td>
<td>8 g over 48 h, oral</td>
<td>Increased by 9% in striatum</td>
</tr>
<tr>
<td>Fujita et al (2000)</td>
<td>$^{[123]}$Jepidepride</td>
<td>AMPT</td>
<td>5.5 g/70 kg over 37 h, oral</td>
<td>Increased by 13% in temporal cortex</td>
</tr>
<tr>
<td>Verhoeff et al (2001)</td>
<td>$^{[11]}$Craclopride</td>
<td>AMPT</td>
<td>4.5 g over 25 h, oral</td>
<td>Increased by 18% in striatum</td>
</tr>
</tbody>
</table>
Table 1-2. Continued...

<table>
<thead>
<tr>
<th>Study</th>
<th>Radioligand</th>
<th>Challenge</th>
<th>Dose and route</th>
<th>Effect on binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verhoeff et al (2002)</td>
<td>$^{11}$Craclopride</td>
<td>AMPT</td>
<td>5250 mg over 29 h, oral</td>
<td>Increased by 12.9% in striatum</td>
</tr>
<tr>
<td>Verhoeff et al (2003)</td>
<td>$^{11}$Craclopride</td>
<td>AMPT</td>
<td>5250 mg over 29 h, oral</td>
<td>Increased by 13.3% in striatum</td>
</tr>
<tr>
<td>Riccardi et al (2008)</td>
<td>$^{18}$Ffallypride</td>
<td>AMPT</td>
<td>71.4 mg/kg over 26 h, oral</td>
<td>Increased by 9–11% in striatal subdivisions and 12.7% in SN</td>
</tr>
</tbody>
</table>

AMPT, α-methyl-para-tyrosine; SN, substantia nigra; ctx, cortex
Table 1-2 lists dopamine release studies in healthy humans that have used pharmacological challenges that directly augment synaptic levels of dopamine, such as amphetamine and methylphenidate. It should be noted that indirect pharmacological challenges, that is, challenges that act on other neurotransmitter systems and indirectly modulate the dopamine system have also been used to estimate striatal dopamine levels of healthy subjects. Challenges that indirectly stimulate dopamine transmission and decrease striatal $[^{11}\text{C}]$raclopride binding include a cholinergic antagonist, scopolamine (Dewey et al 1993), an N-methyl-D-aspartate (NMDA) antagonist, ketamine (Breier et al 1998; Smith et al 1998), and stimulation of 5-HT transmission with fenfluramine (Smith et al 1997) and a 5-HT$_{2A}$ agonist, psilocybin (Vollenweider et al 1999). The use of indirect challenges will not be discussed further in this thesis but does illustrate novel approaches to examining aspects of neurotransmitter interactions with PET. In addition to exploring indirect pharmacological challenges to modulate dopamine levels, recent studies have evaluated the feasibility of non-pharmacological (i.e. behavioural, motor) challenges to induce dopamine release to an extent capable of causing a measurable drop in radioligand binding. Several studies support the feasibility of this approach, demonstrating under specific conditions striatal dopamine release in response to simple motor performance (Goerendt et al 2003; Ouchi et al 2002), non-hedonic food stimulation (Volkow et al 2002), placebo treatments associated with expectation of reward (de la Fuente-Fernandez et al 2002; de la Fuente-Fernandez et al 2001b; Kaasinen et al 2004), a card selection task for monetary reward (Zald et al 2004) and a video game (Koepp et al 1998). In particular, cognitive tasks have also recently been used to modulate phasic dopamine release in various cognitive-mediated brain regions, which will be further discussed in Chapter 2 of this thesis.

Table 1-2 also describes studies that have estimated ‘tonic’ dopamine transmission via pharmacologically-induced dopamine depletion in healthy human volunteers. Acute and rapid dopamine depletion has been achieved through inhibition of tyrosine hydroxylase (via AMPT), a method which will be further described in Chapter 4 of this thesis (General Methods). To date, such studies have demonstrated significant AMPT-induced increases in striatal binding of $[^{123}\text{I}]$IBZM (Abi-Dargham et al 2000b; Laruelle et al 1997a) $[^{11}\text{C}]$raclopride (Verhoeff et al 2003; Verhoeff et al 2002; Verhoeff et al 2001) and recently, $[^{18}\text{F}]$fallypride (Riccardi et al 2008) compared to baseline (no challenge) conditions. Mean percent increase in striatal binding has varied greatly across studies,
ranging between about 9 and 28% (see Table 1-2), which may be due to multiple factors including dose of AMPT, level of dopamine depletion and sample characteristics. Similar to stimulant-induced dopamine release, baseline dopamine D2 receptor occupancy has been estimated in some, but not all, extrastriatal regions using high-affinity D2 radioligands. Following AMPT, [123I]epidepride binding significantly increased by 13% in the temporal cortex (Fujita et al 2000), while [18F]fallypride binding significantly increased by 12.7% in the substantia nigra, over the ~10% increase in striatal subdivisions (Riccardi et al 2008). AMPT-induced increases of radioligand binding have correlated with a decrease in mood (Fujita et al 2000; Laruelle et al 1997a), and attention (Verhoeff et al 2001), as well as cognitive attentional processes (Verhoeff et al 2001) (see Chapter 2). In addition to the acute AMPT method, dopamine depletion has also been achieved through acute tyrosine/phenylalanine depletion (TPD), a dietary manipulation which reduces the availability of the amino acids tyrosine and phenylalanine in order to experimentally reduce the synthesis and release of brain dopamine (McTavish et al 1999; Milner and Wurtman 1986; Tam and Roth 1997). With PET and TPD - achieved via administration of an amino acid drink free of these dopamine precursors, a small (6%), but significant, increase of [11C]raclopride binding was detected in the striatum compared to a balanced amino acid drink including these amino acids (Montgomery et al 2003). Although this finding demonstrates that a dietary manipulation can also influence extracellular dopamine levels, the effect of TPD on brain dopamine concentration may be too small and variable (see Mehta et al 2005) to be reliably used to estimate baseline dopamine receptor occupancy with in vivo binding competition techniques.

Imaging of intra-synaptic dopamine transmission in clinical populations (schizophrenia and PD)

The effect of stimulant and depletion-induced dopamine concentration on in vivo D2 radioligand binding has been evaluated in patients with schizophrenia and PD. Table 1-3 presents those studies that have compared [11C]raclopride or [123I]IBZM binding following pharmacological challenges in both patients and controls. In agreement with the classic dopamine hypothesis of schizophrenia, three studies have reported increased amphetamine-induced “phasic” dopamine release in the striatum of drug-free schizophrenia patients compared to healthy controls, as indexed by displacement of [123I]IBZM (Abi-Dargham et al 1998; Laruelle et al 1996) and [11C]raclopride (Breier et
al 1997) binding. Further, the magnitude of striatal radioligand displacement in patients was correlated with a transient increase in positive symptoms, supporting the view that striatal dopamine overactivity particularly relates to the psychotic phenomena in schizophrenia. Elevated striatal dopamine transmission was also observed in patients with schizotypal personality disorder, similar in magnitude to schizophrenia patients in remission (Abi-Dargham et al 2004), indicating a trait component of dopamine dysregulation within the schizophrenia spectrum disorders. In addition to exacerbated “phasic” dopamine release, schizophrenia patients have also shown higher basal/tonic synaptic dopamine levels, as indexed by greater AMPT-induced increases of striatal $[^{123}\text{I}]$IBZM binding due to a larger “unmasking” of striatal D$_2$ receptors (Abi-Dargham et al 2000b).

**Table 1-3.** PET and SPECT studies of intra-synaptic dopamine transmission in clinical populations compared to controls using pharmacological challenges

<table>
<thead>
<tr>
<th>Study</th>
<th>Radioligand</th>
<th>Challenge</th>
<th>Condition</th>
<th>Effect on in vivo binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laruelle et al (1996)</td>
<td>$[^{123}\text{I}]$IBZM</td>
<td>Amphetamine</td>
<td>Schizophrenia</td>
<td>Increased effect in patients compared to controls</td>
</tr>
<tr>
<td>Breier et al (1997)</td>
<td>$[^{11}\text{C}]$raclopride</td>
<td>Amphetamine</td>
<td>Schizophrenia</td>
<td>Increased effect in patients compared to controls</td>
</tr>
<tr>
<td>Abi-Dargham et al (1998)</td>
<td>$[^{123}\text{I}]$IBZM</td>
<td>Amphetamine</td>
<td>Schizophrenia</td>
<td>Increased effect in patients compared to controls</td>
</tr>
<tr>
<td>Piccini et al (2003)</td>
<td>$[^{11}\text{C}]$raclopride</td>
<td>Methamphetamine</td>
<td>Parkinson Disease</td>
<td>Decreased effect in patients compared to controls</td>
</tr>
<tr>
<td>Abi-Dargham et al (2004)</td>
<td>$[^{123}\text{I}]$IBZM</td>
<td>Amphetamine</td>
<td>Schizotypal personality disorder</td>
<td>Increased effect in patients compared to controls</td>
</tr>
<tr>
<td>Koochesfahani et al (2006)</td>
<td>$[^{11}\text{C}]$raclopride</td>
<td>Methylphenidate</td>
<td>Parkinson Disease</td>
<td>No effect in patients and controls</td>
</tr>
<tr>
<td>Abi-Dargham et al (2000b)</td>
<td>$[^{123}\text{I}]$IBZM</td>
<td>AMPT</td>
<td>Schizophrenia</td>
<td>Increased effect in patients compared to controls</td>
</tr>
</tbody>
</table>

To date, only two studies have compared stimulant-induced dopamine release between PD patients and controls (see Table 1-3). Not surprisingly, methamphetamine-induced putaminal dopamine release was significantly reduced in advanced PD patients compared to matched normal controls, as reflected by a 6.8% displacement of
[11C]raclopride in PD patients compared to a 25% displacement in controls (Piccini et al 2003). In patients, putamen dopamine release was also correlated with [18F]FDOPA uptake, indicating that dopamine release in the putamen is associated with dopamine storage capacity. Further, these results demonstrate that dopamine release induced by pharmacological challenge can still be detected in the putamen of advanced PD patients.

In contrast, Koochesfahani et al (2006) failed to observe an effect of oral methylphenidate on in vivo [11C]raclopride binding in both PD patients (of moderate to advanced disease) and healthy controls. Although this lack of effect in patients is reasonable given the severe dopamine neuron degeneration and loss of DAT occurring in PD, the failure of methylphenidate to displace [11C]raclopride binding in controls is surprising, and inconsistent with a previous study using oral methylphenidate (Volkow et al 2001). Although comparisons with normal controls were not made, several studies have demonstrated the ability for L-dopa (both intravenous and oral) to increase striatal dopamine concentration in PD patients of various stages and disability (de la Fuente-Fernandez et al 2001a; de la Fuente-Fernandez et al 2004; Pavese et al 2006; Tedroff et al 1996b). While L-dopa-induced dopamine release (indicated by reduced [11C]raclopride binding) is associated with clinical improvement in rigidity and bradykinesia (Pavese et al 2006), large (and rapid) increases of synaptic dopamine levels following L-dopa administration appears to contribute to the motor complications, especially dyskinesia, seen in PD (de la Fuente-Fernandez et al 2001a; de la Fuente-Fernandez et al 2004; Pavese et al 2006).

Intra-synaptic dopamine release with in vivo imaging has been evaluated in various other psychiatric disorders including cocaine abuse (Volkow et al 1997), bipolar disorder (Anand et al 2000) and unipolar depression (Parsey et al 2001). Although these studies will not be addressed in this review, they do indicate the wide application of this in vivo imaging technique for assessment of dopamine transmission in a variety of human disorders.

**Antagonist versus agonist radioligands and in vivo vulnerability to endogenous dopamine competition**

The studies presented above all used antagonist D2 radioligands (e.g. [11C]raclopride) to estimate changes in radioligand binding following acute pharmacological challenges. As mentioned previously however, the affinity states of the dopamine D2 receptor are
regulated by G-proteins (Wreggett and Seemn 1983). In general, agonists (such as endogenous dopamine) predominantly label the high-affinity state (Dubois et al 1986; Sibley et al 1982) while antagonists label both high- and low-affinity state receptors (Severson and Randall 1985; Sibley et al 1982). Because of this, researchers have proposed for some time that in vivo imaging results of binding competition would be different between agonist and antagonist ligands (see Fujita and Innis 2002; Laruelle 2000), as the endogenous agonist, dopamine, would be expected to compete more efficiently with agonist compared to antagonist binding as they both preferentially label D₂ receptors in the high-affinity configuration. Recently, this hypothesis has been tested with two D₂ agonist radioligands [11C]NPA and [11C]MNPA in co-injunction with [11C]raclopride. As expected, the agonist ligands were more sensitive to endogenous dopamine competition, showing greater amphetamine-induced displacement than [11C]raclopride in non-human primate brain (Narendran et al 2004; Seneca et al 2006). This greater vulnerability of agonist as compared to antagonist D₂ radioligands suggest that D₂ agonist ligands may be superior for probing intra-synaptic dopamine release as well as the high-affinity state of the D₂ receptor. Nevertheless, this greater in vivo sensitivity of D₂ agonist ligands to acute dopamine manipulations need be confirmed in humans. Further, due to high non-specific (background) binding, the currently available D₂ agonist radioligands [11C]NPA and [11C]MNPA allow assessment in only striatal regions and thus, extrastriatal intra-synaptic dopamine release, at present, cannot be evaluated with these ligands.

Difficulties associated with interpretation of intra-synaptic dopamine release: in vivo confounding factors

The studies described above of intrasynaptic dopamine levels measured by D₂ radioligand displacement and “unmasking” have been exciting, in part because they purport to isolate one component of dopamine function. More specifically however, this neuroimaging measurement of dopamine turnover reflects the net effect of dopamine release and reuptake – as well as the amount of transmitter that diffuses to the D₂ receptor, whether located within or adjacent to the synapse. Thus, the interpretation of the imaging measurement is likely more complex than a simple “increase” or “decrease” in dopamine release as described above. In addition, there are a several other factors – both technical and theoretical, which complicate the interpretation of these in vivo binding competition results. A), the time course of stimulant-induced dopamine release
is much faster (peaking at 10 – 20 min with subsequent decline) than the imaging measurement (an integrated measure over 0.5 to 2 h). Because of this difference in time course, the sensitivity of the imaging results are 10–100 fold lower than direct measurements of extracellular dopamine with microdialysis. For example, Breier et al (1997) found that i.v. amphetamine caused a 21% decrease of $[^{11}\text{C}]$raclopride binding in monkey striatum while concurrent microdialysis showed a 1400% increase in extracellular dopamine. Nevertheless, these measurements were linearly correlated, indicating that D$_2$ radioligand displacement is a “low-gain” monitor of the increase in extracellular dopamine (Fujita and Innis 2002). This apparent error, or “low-gain” monitoring of the imaging measurement may be caused by its poor temporal resolution. Methods that change and maintain new synaptic dopamine levels for 1−2 hours (e.g. with sustained drug-induced dopamine depletion) should be more amenable to accurate imaging measurements.

B), a general problem with the interpretation of dopamine depletion-induced “unmasking” of D$_2$ receptors in humans relates to the difficulty in determining whether dopamine depletion is essentially complete, and thus, whether differences in “unmasking” reflect differences in baseline dopamine occupancy or the extent of dopamine depletion. C), the imaging protocols are vulnerable to technical errors of all radioligand studies to distinguish central versus peripheral effects. For example, do the changes in brain measurement after drug challenge merely reflect a peripheral action on metabolism of the tracer or alterations in regional cerebral flow and delivery of the tracer to the brain? Of note however, is that most compartmental or kinetic models for radioligand quantification control for peripheral effects such as blood flow on the imaging measurement. D), although the imaging studies are intended to measure changes in intrasynaptic dopamine, the D$_2$ receptors themselves are almost certainly altered by the pharmacological challenge. Like most G-protein coupled receptors, agonist binding to the D$_2$ receptor causes uncoupling, internalization, and possibly dimerization (Goggi et al 2007; Rios et al 2001), and in large part, we do not know the effects of this receptor trafficking on the imaging measurement (however see below for discussion of agonist-induced D$_2$ receptor internalisation). E), finally, D$_2$ receptors are not only located synaptically, but also extrasynaptically (Khan et al 1998; Yung et al 1995), and therefore the imaging measurement likely does not solely reflect synaptic changes in dopamine concentration.
*Alternative interpretation of stimulant-induced change in radioligand binding: The Internalization Model*

As previously mentioned, assessment of changes in synaptic dopamine levels within the framework of the occupancy model is based on the assumption that dopamine exerts a purely competitive inhibition on radioligand binding. Although this model is widely accepted, several data are inconsistent with this framework, and point to non-competitive interactions with dopamine to account for radioligand binding changes (Laruelle 2000). Such discrepancies include the paradoxical or unaffected response of butyrophenone compounds to fluctuations in dopamine concentration (Bischoff et al 1991; Chugani et al 1988; Inoue et al 1991; Kobayashi et al 1995), and observations of a prolonged and stable effect of amphetamine on radioligand binding measurements as compared to its short-lived effect on extracellular dopamine levels (Cardenas et al 2004; Laruelle et al 1997b). An alternative framework to the occupancy model that may account for these discrepancies, as well as explain the pharmacologically-induced changes in benzamide radioligand binding described above, is the internalization model.

As mentioned above, agonists such as dopamine typically cause internalisation of G-protein coupled receptors from the cell membrane to the endosomal compartment (Faure et al 1995; Maloteaux and Hermans 1994; Mantyh et al 1995; Sternini et al 1996), an effect that has been documented with D₂ receptors in response to an agonist challenge (Barbier et al 1997; Goggi et al 2007; Ito et al 1999a; Iwata et al 1999; Ko et al 2002; Macey et al 2004; Vickery and von Zastrow 1999). A central characteristic of the internalization model assumes that radioligands are able to bind to the internalized receptor and that specific agents differ in this ability. Benzamides such as raclopride are hypothesised to preferentially bind to the externalized compartment, whereas butyrophenones such as spiperone have access to both externalized and internalized receptors. Furthermore, access to the internalized compartment may result in endosomal trapping and subsequent accumulation of the radioligand (Laruelle 2000). As such, the internalisation model (Figure 1-9) proposes that dopamine released from amphetamine shifts D₂ receptors from the externalized to the internalized compartment, and certain radioligands (i.e. those with low lipophilicity such as benzamide-raclopride) lose access to the internalized receptors which subsequently translates into reduced radioligand binding, whereas others (like spiperone) having access to both compartments show either no change or an apparent increase in binding due to subsequent trapping (Chugani...
et al. 1988; Laruelle 2000; Sun et al. 2003). In contrast, dopamine depletion shifts D$_2$ receptors to the externalised cell membrane, which can be accessed by both butyrophenone and benzamide ligands (Laruelle 2000).

![Image removed for copyright protection – see printed version](image)

**Figure 1-9.** Simplified illustration of the ‘internalization model’. This model proposes that depletion and stimulation of dopamine shifts D$_2$ receptors to the externalised and internalised compartment, respectively. While raclopride and spiperone can both bind to externalised receptors, this model postulates that benzamide-raclopride loses access to internalised D$_2$ receptors, leading to a reduction in raclopride binding. Figure reproduced from Laruelle (2000).

Although in theory the internalisation model may explain the behaviour of different radioligands (i.e. benzamide-raclopride reductions and butyrophenone-spiperone increases) to acute dopamine stimulation, there is little experimental evidence to support it. Chugani and colleagues (1988) were the first to propose the mechanism of spiperone-D$_2$ receptor internalization and subsequent endosomal trapping to account for the paradoxical increases of spiperone binding after stimulant challenge. Further, in a direct comparison of [$^3$H]raclopride and [$^3$H]spiperone binding after amphetamine challenge in rat striatum, Sun et al. (2003) demonstrated that amphetamine-mediated sequestration of D$_2$ receptors are inaccessible to [$^3$H]raclopride but not [$^3$H]spiperone binding. Nevertheless, *in vivo* assessment of amphetamine-induced [$^{11}$C]raclopride binding reductions have not solely been attributed to non-competitive mechanisms, inducing a
change in both D₂ receptor density and affinity (Ginovart et al 2004) or in apparent affinity only (Doudet and Holden 2003). Although further examination of receptor cell trafficking on in vivo PET and SPECT radiotracer binding is clearly warranted, due to the present lack of supportive data the current thesis will work within the framework of the occupancy/competition model described above.
Chapter Two

2 Molecular imaging of the dopamine system and its association with human cognitive function

2.1 INTRODUCTION AND BACKGROUND

As mentioned in Chapter 1, dopamine is a relatively new neurotransmitter, having been discovered within the CNS only 50 years ago. During the last 50 years of dopamine research, the application of new research methodologies has helped increase our understanding of the functional role of dopamine in various diseases, as well as a variety of motor and non-motor behavioural processes. Chapter 1 introduced molecular imaging technology for assessing the dopamine system in vivo in human brain, describing the dopamine radioligands available for human use and some of the clinical findings of the technology in diseases with dopamine alteration as well as healthy aging. This chapter will review a recent application of molecular imaging methods to investigate the role of dopamine in human cognitive function. Contents from this chapter have been published as an invited review in the journal “Biological Psychiatry” (see Appendix 1 for reprint).

2.1.1 Dopamine and cognition: key animal and human studies

There is abundant research implicating the dopaminergic system in cognitive processes, particularly those understood to be subserved by the frontal cortex, striatum and associative structures (frontostriatal or fronto-striato-thalamic circuits, refer to Chapter 1). Such relations have largely been obtained by experimental studies manipulating the dopamine system in animals and humans, as well as clinical observations of cognitive deficits in disorders such as PD and schizophrenia. Certainly one of the most established findings is that of dopamine’s role in working memory, specifically in relation to prefrontal dopamine and D1 receptors. Pioneering work by Goldman-Rakic and colleagues reported that depletion of dopamine by 6-hydroxydopamine in the area surrounding the principal sulcus impaired performance on a delayed alternation task of
working memory in monkeys, mirroring deficits produced by surgical ablation to the same area (Brozoski et al 1979). This deficit was pharmacologically reversed by the dopamine agonists L-dopa or apomorphine, and also replicated in a later study (Roberts et al 1994). Subsequent studies in monkeys and rodents further expanded on such findings, demonstrating a preferential role for PFC D_1 receptors (via administration of dopamine receptor antagonists and agonists) in the modulation of working memory performance and associated PFC neuronal activity (Arnsten et al 1994; Arnsten and Goldman-Rakic 1998; Cai and Arnsten 1997; Sawaguchi and Goldman-Rakic 1991; Sawaguchi and Goldman-Rakic 1994; Seamans et al 1998; Williams and Goldman-Rakic 1995; Zahrt et al 1997). Importantly, these studies demonstrated an inverted ‘U’ dose-dependent relationship, such that an optimal level of D_1 receptor stimulation was needed for optimal function, with both excessive and insufficient D_1 receptor stimulation impairing neuronal delay-associated “memory fields” and, in turn, working memory performance (Goldman-Rakic 1996; Goldman-Rakic et al 2000; Williams and Goldman-Rakic 1995). This inverted ‘U’ concept of catecholamine signaling has since been proposed to mediate prefrontal cortical function and other associated cognitive abilities, and is a central component of frameworks relating baseline dopamine activity to the variable effects of drugs on cognitive functioning (Arnsten and Li 2005; Cools 2006; Mattay et al 2003).

Despite the majority of animal work focusing on working memory, more recent studies in rodents and primates have demonstrated nigrostriatal and mesocortical dopamine modulation of other higher order cognitive processes, including attentional processes, cognitive flexibility, learning and decision making (see reviews by Chudasama and Robbins 2006; Floresco and Magyar 2006). For instance, striatal dopamine transmission has been shown to be important in learning, working memory and certain attentional functions (Collins et al 2000; Crofts et al 2001; Young et al 1998), while PFC dopamine may play a particular role in the acquisition of an attentional set (Crofts et al 2001). In relation to specific dopamine receptors, findings in rats suggest involvement of D_1 (but not D_2) PFC receptors in sustained attention depending on baseline performance (Granon et al 2000), whereas both D_1 and D_2-like PFC receptors have been implicated in cognitive flexibility (on a measure of attentional set-shifting) (Floresco et al 2006; Ragozzino 2002). Cooperative interactions between D_1 and D_2 receptors have been suggested to facilitate set-shifting processes (Floresco and Magyar 2006).
In humans, evidence of a role of dopamine in cognitive processes has been provided by clinical, pharmacological challenge and functional imaging studies. In particular, naturalistic and experimental studies in PD patients have demonstrated the importance of dopamine within the frontostriatal circuits for frontal, executive-type cognitive processes. Although numerous studies have observed cognitive impairment in idiopathic PD across several domains (Nieoullon 2002), impairment is seen most prominently in processes thought to be reliant on the DLPFC and dorsal sectors of the caudate nucleus, such as attentional set-shifting (or cognitive flexibility), planning, and working memory, and represent those seen in patients with frontal lobe damage (Brown and Marsden 1988; Cools 2006; Cooper et al 1991; Dubois and Pillon 1997; Owen et al 1992; Owen et al 1993). Executive dysfunction, particularly in set-shifting processes but also planning, is not only evident in later stage patients, but also in patients early in the course of the disease, when dopaminergic degeneration is largely restricted to the nigrostriatal system (Owen et al 1992; Owen et al 1995). Comparison of medicated and unmedicated patients at different stages of PD has also demonstrated that the deterioration of these processes progress in parallel with the motor deficits that characterize the disorder (Owen 2004; Owen et al 1992). Broad support for a dopaminergic basis is provided from controlled L-dopa treatment and withdrawal studies, which have shown that certain cognitive functions (mainly functions demanding cognitive flexibility) benefit from L-dopa treatment (e.g. Bowen et al 1975; Lange et al 1993) but worsen following withdrawal (Cools et al 2001; Cools et al 2003; Hayes et al 1998; Lange et al 1992). Although certain tasks (e.g. reversal and associative learning) have shown deleterious effects of L-dopa treatment (Cools et al 2001; Gotham et al 1988), this has been attributed to an “overdosing” effect of dopamine within less affected striatal circuits (i.e. ventral striatum frontostriatal circuits). As such, the pattern and progression of cognitive deficits and the contrasting effects of medication seen in PD has been proposed to depend on the nature of the task and the basal level of dopamine within underlying frontostriatal circuits (see Cools 2006; Owen 2004).

Pharmacological manipulation of dopamine transmission in healthy volunteers has also been shown to modulate performance on executive tasks such as working memory, planning, attentional set-shifting and attentional control. The majority of research has focused on direct D₂ receptor agonists or catecholamine stimulants and their effects on
working memory. Despite the animal literature suggesting critical involvement of PFC $D_1$ receptors in executive processes, this has been difficult to verify in humans owing to the lack of pharmacological agents that can selectively probe the $D_1$ receptor in human subjects. Systemic administration of low doses of $D_2$ receptor agonists (such as bromocriptine) has improved performance on certain cognitive tasks, particularly tasks of working memory but also response inhibition (stroop) and memory span (Luciana and Collins 1997; Luciana et al 1998; Mehta et al 2001; Roesch-Ely et al 2005). Dopaminergic stimulants such as methylphenidate and amphetamine have likewise demonstrated some beneficial cognitive effects on attentional processes, working memory and to a lesser extent, set-shifting and planning (see Mehta and Riedel 2006). Further, facilitatory effects of dopaminergic agonists may be more pronounced, or only evident, in individuals with low baseline cognitive performance (Kimberg et al 1997; Mattay et al 2000; Mehta et al 2000), although not all studies have confirmed an effect of agonists on working memory or demonstrated baseline dependency. Inconsistent findings of dopamine agonists on cognition are also evident, which have been attributed to differences in tasks and task parameters, pharmacological doses and individual variation in baseline performance or basal dopamine levels, as reviewed elsewhere (Ellis and Nathan 2001; Mehta and Riedel 2006). In comparison, pharmacological reduction in dopamine transmission via $D_2$ receptor antagonists (such as sulpiride or haloperidol) and depletion of the dopamine precursors tyrosine and phenylalanine has impaired performance on some “frontal lobe” cognitive tasks (e.g. Harmer et al 2001; Harrison et al 2004; Mehta et al 2004; Mehta et al 1999). However, although these studies suggest dopaminergic regulation of various human cognitive processes, the anatomical regions and the specific dopaminergic mechanisms that mediate such actions cannot be identified.

Studies of regional cerebral blood flow following dopamine stimulants in healthy subjects have delineated particular working memory-related brain circuits that are modulated by dopamine (Mattay et al 2000; Mehta et al 2000). For instance, methylphenidate-induced improvements in working memory performance was associated with task-related reductions in blood flow in the DLPFC and posterior parietal cortex (Mehta et al 2000). In addition, amphetamine was demonstrated to increase blood oxygen level dependent signal within the PFC during working memory performance, although the magnitude of this change was smaller in subjects who
behaviourally improved from the drug (only those with low baseline performance) than individuals who showed deleterious behavioural effects (Mattay et al 2000). These findings have been interpreted to represent more efficient brain processing (i.e. less drug induced activation with improved performance). Although such functional imaging studies define the neuroanatomical loci of drug-induced behavioural effects, they do not directly measure dopaminergic indices and their correlates with cognition. Knowledge of the latter is important in determining the anatomical regions that are critical to cognitive processes modulated by the dopamine system.

### 2.1.2 Using molecular imaging to explore the relationship between dopamine and human cognition

As detailed in Chapter 1, molecular imaging allows the direct assessment of components of the dopamine system by selectively imaging dopamine receptors, transporters or enzymes. An advantage of molecular imaging technology over other methodologies is that different dopamine targets can be examined and quantified in living, human subjects. Because of the large number of radiotracers that can examine pre-, intra-, and post-synaptic components of the dopamine system (reviewed in Chapter 1), and because of the strong experimental and clinical evidence, as described above, linking dopamine and cognition, molecular imaging techniques have recently examined relationships between molecular markers of the dopamine system and cognitive processes in normal subjects and patient populations. The section presented below will review molecular imaging studies that have explored the relationship between cognitive processes and components of the dopamine system (pre-, post and intra-synaptic) in healthy human subjects and several patient groups with known or hypothetical dopamine and cognitive dysfunction, such as PD, schizophrenia and Huntington’s disease. Like Chapter 1, this section will cover studies both within and outside the striatum, with a particular focus on the frontostriatal circuitry. Some of the cited studies have been described in Chapter 1 but without review of the cognitive correlates. As mentioned previously, an earlier version of this review was published in Biological Psychiatry (Cropley et al 2006a) (see Appendix 1 for reprint).
2.2 PRE-SYNAPTIC DOPAMINE MODULATION OF COGNITIVE FUNCTION

2.2.1 Striatal $[^{18}]$FDOPA studies in Parkinson disease

As mentioned in Chapter 1, $[^{18}]$FDOPA PET is a useful tool to quantify the loss of nigrostriatal dopamine terminal function in PD. $[^{18}]$FDOPA uptake not only measures dopamine synthesis capacity (by AADC), but the storage of fluorodopamine within pre-synaptic dopamine terminals. Striatal $[^{18}]$FDOPA measurements are reliably associated with motor impairment and have been used to monitor disease severity and progression over time (see Chapter 1). However, the clinical manifestations of PD are not only restricted to motor deterioration. Cognitive impairment, usually described as frontostriatal in nature, as well as dementia, are also common (Aarsland et al 2003; Zgaljardic et al 2003). The capability of $[^{18}]$FDOPA PET to measure the loss of presynaptic dopamine terminals places it in a prime position to investigate the cognitive consequences of dopamine deficiency.

Several studies have directly assessed the relationship between dopaminergic denervation and cognitive performance in PD using $[^{18}]$FDOPA (see Table 2-1). Many of these studies also tested the assertion that the putamen is more important for motor function (primarily being part of the “motor loop”), while the caudate nucleus is more important for behavioral, including cognitive functions (primarily involved in the ‘prefrontal loop’ (Alexander and Crutcher 1990). To support this, correlations have been reported between reduced $[^{18}]$FDOPA uptake in the caudate nucleus and cognitive dysfunction. $[^{18}]$FDOPA reductions have been associated with; 1) impaired verbal episodic memory in advanced PD (Holthoff-Detto et al 1997) and twins discordant for PD (Holthoff et al 1994), 2) with impairment in a tactile object discrimination task in non-demented, medication withdrawn PD (Weder et al 1999), 3) with impairment in attentional and inhibitory functioning in early or heterogeneous (early to late) PD (Bruck et al 2001; Rinne et al 2000) and 4) with worse composite scores in memory, executive functioning and fluency domains in advanced PD (van Beilen et al 2008). These findings are consistent with reports of explicit memory and executive deficits in PD (Dubois and Pillon 1997) and the demonstration that executive dysfunction in PD is accompanied by reduced activity within the caudate nucleus and prefrontal regions (Lewis et al 2003). Such associations between pre-synaptic dopamine denervation and certain executive deficits suggest that impaired caudate dopaminergic
transmission may contribute to the cognitive deficits of patients. With the exception of two studies (Holthoff et al 1994; van Beilen et al 2008), \([^{18}F]\)FDOPA reductions in the putamen were associated with motor disability only and not cognitive impairment. Correlations between cognitive tasks and putamen \([^{18}F]\)FDOPA in PD (Holthoff et al 1994; van Beilen et al 2008) indicate that dopaminergic mechanisms within the putamen contribute, in part, to cognitive processes, despite its emphasis on motor function. A recent study (van Beilen et al 2008) reported that \([^{18}F]\)FDOPA uptake in both the putamen and caudate of advanced PD was related to executive functions emphasizing cognitive flexibility, while caudate \([^{18}F]\)FDOPA was also related to memory and fluency variables alleged to involve behavioural organization. The authors proposed that the caudate may be more important for the “mental” components of executive functioning, while the putamen more important for the “motor” components of executive functions (van Beilen et al 2008). However, various methodological limitations, including measurement of \([^{18}F]\)FDOPA in patients on medication, lack of dynamic kinetic modeling of \([^{18}F]\)FDOPA data (i.e. use of a ratio method from a single static image) and no control of multiple comparisons requires that these results should be interpreted with caution. Nevertheless, these striatal correlations are supported by a recent study in healthy individuals showing correlations between \([^{18}F]\)FDOPA influx (from full linear graphical analysis) in the striatum (both caudate and putamen) and tests of “prefrontal function” (sustained attention, stroop and trail-making tasks), although with correction for multiple comparisons, the correlations between putamen \([^{18}F]\)FDOPA and stroop performance only remained significant (Vernaleken et al 2007a). Contrary to the above reports and to the caudate nucleus-cognition or even a striatal-cognition model, three studies have failed to show an association between caudate nucleus (or putamen) \([^{18}F]\)FDOPA uptake and cognitive performance in non-demented PD patients (Broussolle et al 1999; Bruck et al 2005; Nagano-Saito et al 2004). Differences in the clinical features of the PD patients examined (e.g. in disease severity, dementia, etc) and the cognitive tests used make comparisons amongst studies difficult (see below).
2.2.2 Extrastriatal \([^{18}F]FDOPA\) studies in Parkinson disease

Disruptions in the mesocorticolumbic pathways have also been associated with cognitive deficits in PD. For instance, Rinne et al (2000) found an association between reduced \([^{18}F]FDOPA\) uptake in the frontal cortex and deficits in working and immediate memory and executive strategies. However, after statistically controlling for disease duration and severity, some of these relationships failed to reach significance. Several subjects also showed mild cortical atrophy, which could potentially account for the lower \([^{18}F]FDOPA\) uptake in the frontal cortex and cognitive deterioration. In another study, \([^{18}F]FDOPA\) reductions in the anterior cingulate were observed in PD patients with dementia (Ito et al 2002). However, as AADC is also present in noradrenergic and serotonergic neurons (Tison et al 1991) (refer to Chapter 1), the cortical \([^{18}F]FDOPA\) reductions may be a composite of dopaminergic, serotonergic and noradrenergic systems, which also degenerate in advanced PD.

In contrast, early, non-demented and non-medicated PD patients were recently reported to show increased \([^{18}F]FDOPA\) uptake in cortical areas covering the DLPFC, anterior cingulate and medial frontal cortex (Bruck et al 2005), replicating previous observations of increased cortical \([^{18}F]FDOPA\) uptake in early, medicated (Rakshi et al 1999) and unmedicated (Kaasinen et al 2001) PD patients. Although increased cortical \([^{18}F]FDOPA\) uptake is counterintuitive, it may be a compensatory process in the cortical-subcortical dopamine loops (see below). Interestingly, these cortical \([^{18}F]FDOPA\) increases were differentially associated with attentional functioning. In a region encompassing the anterior cingulate and medial frontal cortex, increased uptake was strongly related to decreased Stroop interference time (reflecting decreased processing time to suppress attention), while in the right DLPFC, \([^{18}F]FDOPA\) uptake was positively correlated with reaction time on a vigilance test assessing sustained attention. These results are in accordance with blood flow studies linking sustained attention to the right DLPFC (Cabeza and Nyberg 2000), and suppressed attention to the dorsal anterior cingulate and inferior frontal sulcus (Duncan and Owen 2000). Indeed, greater \([^{18}F]FDOPA\) uptake in the dorsal anterior cingulate has also been associated with reduced interference on the Stroop task in medicated male schizophrenic patients and controls (McGowan et al 2004a), providing further support for the involvement of anterior cingulate dopamine function in suppressed attention/response inhibition.
processes. However, it is not clear why greater DLPFC [$^{18}$F]FDOPA uptake was associated with poorer sustained attention. A possible explanation could be that the effect of dopamine on cognitive performance may be dependant on the nature of the cognitive task (i.e. reflecting specific task demands and different neural substrates), and the basal level of dopamine in the underlying fronto-striato-thalamic circuitry, as has been proposed by others (e.g. Cools 2006; Cools et al 2003).

Finally, [$^{18}$F]FDOPA uptake in only the left hippocampus was positively correlated with Raven’s Colored Progressive Matrices (RCPM), a test reliant on executive and visuospatial functions, in non-demented PD patients (Nagano-Saito et al 2004). It was speculated that the involvement of hippocampal dopamine in RCPM may have represented recruitment of an alternative network to compensate for caudate nucleus dysfunction, a concept supported by a PET activation study in PD patients demonstrating decreased caudate nucleus activation but increased hippocampal activation during a planning task (Dagher et al 2001).

As previously mentioned in Chapter 1, it should be noted that as the [$^{18}$F]FDOPA signal-to-noise ratio is quite low in cortex, it is uncertain whether [$^{18}$F]FDOPA uptake can be reliably quantified in cortical areas. Therefore, the above studies should be viewed as preliminary and require further replication.

### 2.2.3 DAT studies in Parkinson disease

Studies that have measured striatal DAT binding in PD and cognitive function also support an association between pre-synaptic dopamine hypofunction and impairment on executive-type processes (see Table 2-1). In non-demented, medicated PD patients, decreased [$^{11}$C]nomifensine binding in the right caudate nucleus was associated with impairments on the object alternation task, which assesses attentional set-shifting, while a weaker, negative correlation in the bilateral putamen was observed between [$^{11}$C]nomifensine binding and performance on a planning task (Marie et al 1999). The object alternation findings indicate that loss of caudate dopamine terminal function is associated with impaired attentional set-shifting. In the opposite direction, Roberts et al (1994) reported that higher dopamine release in the monkey caudate nucleus was
associated with better set-shifting performance, further suggesting a role of caudate dopamine in set-shifting processes. Using $[^{123}\text{I}]{\beta}\text{-CIT}$ in non-demented PD, decreased DAT uptake in both the caudate nucleus and putamen was associated with impaired performance on the modified card sort test and digit-ordering, representing abilities such as attentional set-shifting, cognitive flexibility and working memory, as well as immediate and delayed recall on the logical memory subtest (Muller et al 2000), although these relationships were relatively weak and may have been a function of age and motor disability. Furthermore, in non-demented PD, deficits in simultaneous processing time (a type of attentional processing demanding a sharing of attention) in unmedicated (OFF) states were associated with lower $[^{123}\text{I}]{\beta}\text{-CIT}$ binding in the left striatum, suggesting that deficits in cognitive tasks that involve a sharing of attention and a simultaneous response may be linked to striatal dopamine depletion and disruption of premotor circuits (Duchesne et al 2002). Deficits in tasks involving concurrent cognitive processing have previously been observed in “off” state and de novo PD patients (Malapani et al 1994), further indicating that adequate levels of dopamine transmission is necessary for simultaneous processing or attention sharing demands. Although these findings of cognitive deficits cannot be attributed to caudate nucleus dopamine dysfunction alone, these DAT findings do suggest that striatal dopamine deficiency may contribute to prefrontal cognitive deficits in PD by disrupting fronto-striato-thalamic loops.

### 2.2.4 DAT studies in healthy aging

A number of molecular imaging studies have demonstrated age-related decreases (on the order of 3 – 8% per decade) in DAT density in healthy subjects (see Chapter 1). These age-related decreases in DAT binding have shown to be a significant mediator of cognitive changes in memory and executive functioning, and cognitive performance in general (see Table 2-1). For instance, Mozley et al (2001) reported age-related reductions of DAT in the putamen and caudate nucleus together with age-related impairments in verbal memory/learning performance. Furthermore, in women, but not men, higher striatal DAT binding was associated with better Stroop performance. In another study, Erixon-Lindroth et al (2005) further corroborated these age-related reductions in DAT density and associations with age-related decline in episodic

---

66
memory and executive functioning. Importantly however, the influence of age on cognitive performance was essentially eliminated following statistical control of DAT availability. Further, the finding of a positive association between DAT and crystallized intelligence, which did not vary with age, indicates a general role of DAT availability in cognitive function, over and above age.
<table>
<thead>
<tr>
<th>Study</th>
<th>Target</th>
<th>Radioligand</th>
<th>Population</th>
<th>Cognitive task(s)</th>
<th>Brain region</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Holthoff et al (1994)</td>
<td>AADC</td>
<td>$[^{18}F]$FDOPA</td>
<td>PD</td>
<td>Selective reminding task</td>
<td>Caudate and putamen</td>
<td>Reduced uptake associated with worse performance</td>
</tr>
<tr>
<td>Rinne et al (2000)</td>
<td>AADC</td>
<td>$[^{18}F]$FDOPA</td>
<td>PD</td>
<td>Digits span (backwards), verbal fluency, verbal immediate recall</td>
<td>Caudate</td>
<td>Reduced uptake associated with worse performance</td>
</tr>
<tr>
<td>Vernaleken et al (2007a)</td>
<td>AADC</td>
<td>$[^{18}F]$FDOPA</td>
<td>Healthy</td>
<td>CPT, Trail-Making Test, Stroop</td>
<td>Caudate, putamen and midbrain</td>
<td>Increased uptake associated with better performance</td>
</tr>
<tr>
<td>Van Beilen et al (2008)</td>
<td>AADC</td>
<td>$[^{18}F]$FDOPA</td>
<td>PD</td>
<td>Composite scores for memory$^a$, executive function$^b$, fluency domains$^c$, composite score for executive function domain$^d$</td>
<td>Caudate</td>
<td>Increased uptake associated with better performance on cognitive domain</td>
</tr>
</tbody>
</table>
Table 2-1. Continued…

<table>
<thead>
<tr>
<th>Study</th>
<th>Target</th>
<th>Radioligand</th>
<th>Population</th>
<th>Cognitive task(s)</th>
<th>Brain region</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marie et al (1999)</td>
<td>DAT</td>
<td>[(^{11}\text{C})]nimodipine</td>
<td>PD</td>
<td>Object alternation</td>
<td>Right caudate</td>
<td>Reduced binding associated with worse performance</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Conditional associative learning</td>
<td>Putamen</td>
<td>Increased binding associated with worse performance</td>
</tr>
<tr>
<td>Muller et al (2000)</td>
<td>DAT</td>
<td>[(^{123}\text{I})]CIT</td>
<td>PD</td>
<td>Card sorting test, digit ordering</td>
<td>Caudate and</td>
<td>Reduced binding associated with worse performance</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>test, logical memory</td>
<td>Putamen</td>
<td></td>
</tr>
<tr>
<td>Mozley et al (2001)</td>
<td>DAT</td>
<td>[(^{99}\text{mTc})]TRODAT-1</td>
<td>Healthy</td>
<td>Pennsylvania verbal learning test</td>
<td>Caudate and</td>
<td>Better performance associated with higher binding</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Stroop</td>
<td>Putamen</td>
<td>Greater RT associated with lower DAT binding (women only)</td>
</tr>
<tr>
<td>Duchesne et al (2002)</td>
<td>DAT</td>
<td>[(^{123}\text{I})]CIT</td>
<td>PD</td>
<td>Simultaneous processing task</td>
<td>Left striatum</td>
<td>Lower binding associated with longer processing</td>
</tr>
<tr>
<td>Erixon-Lindroth et al</td>
<td>DAT</td>
<td>[(^{11}\text{C})]β-CIT-FE</td>
<td>Healthy</td>
<td>Word recall, R-OCF, face recognition, visuospatial WM test, COAT, Information subtest from WAIS-R</td>
<td>Striatum</td>
<td>Lower binding associated with worse performance</td>
</tr>
</tbody>
</table>

PD, Parkinson’s disease patients; R-OCF, Rey-Osterrieth’s Complex Figure; WM, working memory; COAT, Controlled Oral Association Test; AC, anterior cingulate; AC-MFC, anterior cingulate and medial frontal cortex; DLPFC, dorsolateral prefrontal cortex; CPT, continuous performance test; RT, reaction time; \(^{a}\) memory domain aggregate of scores from the memory scale of the Mattis Dementia Rating Scale and 15 Word Tests learning and recall scores; \(^{b}\) executive functioning domain aggregate of scores from Odd Man Out test, Stroop task and Trail making test; \(^{c}\) fluency domain aggregate of scores from categorical and letter fluency tests.
2.2.5 **Overview of pre-synaptic dopaminergic markers and cognitive function**

Studies that have examined pre-synaptic dopamine markers in relation to cognition suggest an important role of dopamine synthesis and uptake in modulation of human cognitive processes. Delineating the specific processes regulated by dopamine, and the precise regions mediating these processes from the above reports however, is difficult due to the different populations examined, and variations within populations. For instance, studies examining PD patients varied on disease severity and duration, degree of depression, whether patients were demented, and the patient’s medication status (i.e. on, off, or de novo). Furthermore, not all studies adequately controlled for the effect of motor symptoms on task performance. Differences in the types of cognitive tests used also add significant variation for study comparisons. Despite these variations, it appears that in PD and healthy aging, changes in striatal and cortical dopamine transmission is associated with memory, learning and a range of prefrontal-type cognitive functions such as response inhibition, attentional set-shifting, working memory and sustained attention. Impairments in episodic memory observed in PD may also be related to executive dysfunction (Higginson et al 2003). Such associations between presynaptic dopamine and frontal-type cognition fits within the existing literature that shows such executive processes to be altered by dopaminergic challenges in healthy subjects (e.g. Harrison et al 2004; Mehta et al 1999; Roesch-Ely et al 2005) and impairment of certain executive processes following L-dopa withdrawal in PD patients (Lange et al 1992).

Due to the reciprocal connections between the basal ganglia, frontal cortex, limbic and brainstem structures, it is difficult to define the specific roles of the striatum and cortex in information processing. Consequently, it is commonly viewed in terms of frontostriatal, or fronto-striato-thalamic functions or circuitry, and disruption in any one component of this circuitry can lead to functional alterations in other parts. Thus, striatal, and in particular caudatal, presynaptic dopamine deficiency in PD and healthy aging likely impairs frontostriatal functioning through disruption of the fronto-striato-thalamic loops, as has been suggested from functional imaging studies in PD (Owen et al 1998). Although the DAT studies generally did not support the segregation of the putamen and caudate nucleus into respective motor and cognitive functions, the relative poor spatial resolution of SPECT (which was commonly used in the DAT studies) compared to PET (which was only used in the \[^{18}F\]FDOPA studies) may have
contributed to the lack of this dissociation. Therefore, fronto-striato-thalamic disruption may occur at the level of the caudate nucleus, rather than putamen. Alterations of presynaptic dopamine in cortical regions such as the anterior cingulate and frontal cortex was also associated with working memory, response inhibition and attentional deficits in PD patients, the mechanisms by which may be a function of disease stage. In early PD, nigrostriatal dopamine depletion may cause a compensatory up-regulation of dopamine brought about by increased AADC activity in the mesocortical system, which may differentially affect some frontal cognitive functions (Bruck et al 2005). In advanced PD however, dopamine deficiency extends to the frontal cortex, possibly due to progressive denervation of the mesocortical dopamine pathway, and may directly interfere with PFC functions such as working memory (Rinne et al 2000). Therefore, both nigrostriatal and mesocortical dopamine alterations may contribute to certain cognitive changes in PD and healthy subjects, either directly, or indirectly through disrupting fronto-striato neuronal loops.

2.3 DOPAMINE RECEPTORS AND COGNITIVE FUNCTION

2.3.1 Dopamine D$_2$ receptors

Experimental and blood flow studies in animals and humans have demonstrated some relationships between D$_2$ receptors, presumably acting within the striatum, and cognitive processes, particularly in switching behavior and aspects of working memory and planning (Arnsten et al 1995; Mehta et al 2003; Mehta et al 1999). Molecular imaging studies have recently explored the influence of striatal D$_2$ receptor changes on cognition in aging and certain neurological and psychiatric disorders (Table 2-2).

Like dopamine transporters, normal age-related loss (around 10% per decade) of dopamine receptors in both striatal (Ichise et al 1998) and extrastriatal (Kaasinen et al 2000b) areas has been reported (refer to Chapter 1 for overview). Consistent with the DAT findings, this loss of striatal D$_2$ receptors (indicated by decreased [$^{11}$C]raclopride binding) in healthy aging is also associated with certain cognitive deficits. Similarly, the deficits have been observed in episodic memory and executive function, but also extend to perceptual speed processes (Backman et al 2000; Reeves et al 2005; Volkow et al 1998a). Mirroring the findings obtained with DAT, D$_2$ receptor loss strongly accounted
for cognitive impairment irrespective of chronological age, although a paradoxical relationship has been observed between higher D₂ binding and worse spatial working memory performance (Reeves et al 2005). However, in general, these results provide further support that dopaminergic neurotransmission contributes to age-related cognitive decline and cognitive variation in general.

Decreases in striatal D₂ receptors may contribute to cognitive impairments in certain neurological and psychiatric disorders. For instance, D₂ receptor reductions, and to a lesser extent D₁ reductions, in the caudate nucleus and putamen, were associated with impairments in a number of executive function tasks measuring planning, memory, response inhibition and sequencing processes in asymptomatic individuals with Huntington’s disease (Lawrence et al 1998). Likewise, in schizophrenic patients, decreased striatal D₂ receptor density was associated with poorer performance on an attentional task involving discriminating targets from non-targets, but only when the effects of time were considered (Yang et al 2004a), suggesting that tasks which involve time constraints or require optimal timing may be related to striatal dopamine activity. However, not all patients were neuroleptic-free, and although dosage of neuroleptic was controlled for in the analysis, the precise nature of the relationship between striatal D₂ receptor changes and the cognitive task cannot be determined. Nevertheless, the notion of optimal timing of processing is worthwhile to explore in light of research relating timing mechanisms to basal ganglia function. It has been suggested that temporal processing of brief intervals is reliant on dopaminergic activity in the basal ganglia (Rammsayer 1999). PD patients, who are characterized by severe nigrostriatal denervation, have also been reported to show temporal processing deficits (Harrington et al 1998). Consistent with this notion, several of the measures that have shown associations with D₂ receptor binding, as well as presynaptic dopamine markers, in the striatum, involve time pressure or are dependent on optimal processing within short durations (such as processing speed tasks and attentional tasks such as the Stroop). In addition, some of the tasks associated with striatal dopamine also rely on suppressing attention to competing information or efficient sequential ordering of responses, fitting with proposals that the basal ganglia acts to inhibit competing actions and is involved in optimal organization of information (Casey et al 2002; Dubois and Pillon 1995; Graybiel 1998; Mink 1996). Associations have also been observed between striatal dopamine changes and tests of episodic, recognition and working memory, which do not
fit within this framework of basal ganglia function. Therefore, while striatal dopamine changes are likely to directly affect some fundamental aspects relating to attentional control and temporal processing, such delineations cannot be clearly determined with these studies. Similar to the preceding section, striatal D\textsubscript{2} receptor alterations may contribute to cognitive changes via demodulation of the fronto-striato-thalamic circuitry, and performance on these tasks should be largely viewed within the context of this circuitry.

Although several radioligands have recently been developed to assess D\textsubscript{2} receptors in extrastriatal areas, to our knowledge only one study has examined the cognitive correlates of extrastriatal D\textsubscript{2} receptors (Lumme et al 2007). This study demonstrated significant positive correlations between $[^{11}\text{C}]$FLB 457 binding in the right anterior cingulate and error parameters (non-perseverative and perseverative) on the Wisconsin card sorting test (WCST) in young healthy individuals. The functional interpretation of this somewhat paradoxical correlation (indicating high D\textsubscript{2} receptor binding in anterior cingulate predicts impaired executive performance) is unclear, but may be related to excessive D\textsubscript{2}-mediated flexibility or in contrast, may be a function of decreased dopaminergic activity (Lumme et al 2007). The associations were relatively weak however, and no corrections for multiple comparisons were made despite numerous regions being sampled, placing the study at risk of producing a false-positive result. Nevertheless, a voxel-based analysis confirmed the finding, giving credence to the association. Further studies examining extrastriatal D\textsubscript{2} receptors are required to elucidate the role of cortical D\textsubscript{2} receptors in the modulation of human cognitive processes.

### 2.3.2 Dopamine D\textsubscript{1} receptors

Over the past decade, converging work in monkeys, neuronal recordings and computational models have indicated the involvement of D\textsubscript{1} receptors in modulating and stabilizing pre-frontal cortical networks and functions. Local application of specific D\textsubscript{1} receptor agonists and antagonists into the PFC of monkeys has mediated spatial working memory performance in a dose-dependant manner (Cai and Arnsten 1997; Sawaguchi and Goldman-Rakic 1991; Sawaguchi and Goldman-Rakic 1994), and via
modulation of pyramidal neurons, or “memory fields” of the prefrontal cortex (Williams and Goldman-Rakic 1995). In humans, administration of a mixed D₁/D₂ agonist, pergolide, has improved working memory performance in some (Muller et al 1998) but not other (Bartholomeusz et al 2003) studies. However, as mentioned previously, investigation of the role of D₁ receptors in human cognitive processes has been limited due to the lack of selective D₁ compounds for human use.

Two PET studies have observed a relationship between D₁ receptor density in the PFC and executive performance in neuroleptic-naïve or -free schizophrenic patients (see Table 2-2). Reports have been conflicting however, with both decreases (Okubo et al 1997) and increases (Abi-Dargham et al 2002) in PFC D₁ receptor binding being associated with impairments in executive functions, as assessed by the WCST and n-back task. The main difference between these PET studies (in addition to the different tasks) is that the former study, showing decreased D₁ receptors, used the D₁ radioligand \([^{11}C]\text{SCH23390}\), while the latter study, reporting increased ligand binding, used the radioligand \([^{11}C]\text{NNC 112}\).

As previously discussed in Chapter 1, both authors claim that their D₁ receptor findings are consistent with an overall hypodopaminergic state in the PFC of schizophrenia, which in turn contributes to executive dysfunction. Thus, Okubo et al (1997) proposed that their finding of decreased \([^{11}C]\text{SCH 23390}\) binding reflects D₁ receptor hypofunction, leading to decreased dopaminergic activity in the PFC and associated cognitive deficits. In contrast, Abi-Dargham et al (2002) proposed that both the increased \([^{11}C]\text{NNC 112}\) binding and the associated working memory deficits are secondary to sustained prefrontal dopamine depletion. Although the reasons for these contradictory results are unknown, an intriguing possibility is that the two PET radioligands (SCH 23390 and NNC 112) respond differentially to dopamine depletion in terms of internalization and availability of binding to the ligand (Guo et al 2003) (see Chapter 1 for further detail). Despite the ambiguity, these PET imaging results support findings obtained in animals suggesting important roles for prefrontal dopamine and D₁ receptors in cognitive function, and indicate a means for selectively examining D₁ receptors in humans, which has previously been difficult due to a lack of appropriate pharmacological agents.
<table>
<thead>
<tr>
<th>Study</th>
<th>Target</th>
<th>Radioligand</th>
<th>Population</th>
<th>Cognitive task(s)</th>
<th>Brain region</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yang et al (2004a) D2</td>
<td>D2</td>
<td>[123I]IBZM</td>
<td>Schizophrenia</td>
<td>Tai-Ta attention test</td>
<td>Striatum</td>
<td>Decreased binding associated with worse performance</td>
</tr>
</tbody>
</table>

HD, Huntington’s disease; TOL, Tower of London; WCST, Wisconsin Card Sort Test; RSPM, Raven’s Standard Progressive Matrices; SDMT, Symbol Digit Modalities Test; CANTAB, Cambridge Neuropsychological Test Automated Battery; SWM, spatial working memory; DLPFC, dorsolateral prefrontal cortex
2.4 DOPAMINE RELEASE AND COGNITIVE FUNCTION

As reviewed in Chapter 1, PET and SPECT can estimate synaptic concentration of endogenous neurotransmitters. In brief, the basis of this approach is that radioligands compete with the endogenous neurotransmitter for binding to receptors. Thus, higher synaptic neurotransmitter concentrations are associated with lower radioligand binding and vice versa (see Chapter 1 for further detail).

Using this technique with benzamide D₂ receptor radioligands (e.g. [¹¹C]raclopride or [¹²³I]IBZM) two types of release can be estimated: 1) “phasic” or stimulant-induced dopamine release, by comparing D₂ radioligand binding at baseline to challenge conditions that increase synaptic dopamine levels, and 2) “tonic” dopamine release, by comparing baseline radioligand binding to transient dopamine depleted conditions. Such increases and decreases in synaptic dopamine levels have induced decreases and increases, respectively, in D₂ binding potential (as reviewed in Chapter 1).

Administration of pharmacological stimulants (e.g. amphetamine or methylphenidate) has typically been used to initiate phasic dopamine release, both in healthy humans and various patient populations. Although previous studies have examined potential relationships between dopamine release and measures of mood and arousal, only one study has correlated stimulant-induced dopamine release with cognitive performance. Riccardi et al (2006) reported several significant correlations between regional amphetamine-induced dopamine release (indexed by [¹⁸F]fallypride displacement) and changes in cognition in healthy subjects. Correlations were apparent in striatal and extrastriatal areas on measures of attentional function, conflict control and processing speed, but not working memory. Specifically, greater dopamine release in the ventral striatum, ventral putamen and basal forebrain was associated with improved processing speed (assessed by digit symbol coding and symbol search tasks), while dopamine release within the lateral and inferior temporal cortices and insula was associated with attention/conflict control (assessed by the stroop). Although alterations in striatal dopamine release have been observed in disorders such as schizophrenia (Breier et al 1997; Laruelle et al 1996), PD (Piccini et al 2003) and chronic cocaine abuse (Volkow et al 1997) using pharmacological challenges, such alterations have not been related to cognitive performance.
Table 2-3. Molecular imaging studies showing associations between dopamine release and cognition in healthy human subjects

<table>
<thead>
<tr>
<th>Study</th>
<th>Target</th>
<th>Radioligand</th>
<th>Challenge</th>
<th>Cognitive task(s)</th>
<th>Brain region</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tonic Release</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mehta et al (2005)</td>
<td>D2</td>
<td>$[^{11}C]$raclopride</td>
<td>TPD</td>
<td>Modified TOL</td>
<td>Dorsal and ventral striatum</td>
<td>Increase in binding correlated with worse performance (dorsal) and faster responses (ventral)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Modified delayed response task</td>
<td>Dorsal striatum</td>
<td>Increase in binding correlated with worse performance</td>
</tr>
<tr>
<td><strong>Phasic Release</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aalto et al (2005)</td>
<td>D2</td>
<td>$[^{11}C]$FLB457</td>
<td>Cognitive</td>
<td>N-back task (0-back)</td>
<td>Left ventral AC</td>
<td>Decreased binding during task performance compared to baseline condition</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N-back task (2-back)</td>
<td>VLFC, left MTC, left ventral AC</td>
<td>Decreased binding during task performance compared to 0-back task, and baseline condition</td>
</tr>
<tr>
<td>Monchi et al (2006a)</td>
<td>D2</td>
<td>$[^{11}C]$raclopride</td>
<td>Cognitive</td>
<td>Montreal card sorting task</td>
<td>Striatum</td>
<td>Decreased binding during planning of set shift compared to matching according to an ongoing rule</td>
</tr>
<tr>
<td>Riccardi et al (2006)</td>
<td>D2</td>
<td>$[^{18}F]$fallypride</td>
<td>Amphetamine</td>
<td>Digit symbol coding, Symbol search task, Stroop task</td>
<td>Ventral striatum, basal forebrain, Ventral putamen, Lateral and inferior temporal cortex, insula</td>
<td>Decrease in binding correlated with better performance</td>
</tr>
</tbody>
</table>

AMPT, alpha-methyl-para-tyrosine; TPD, tyrosine and phenylalanine depletion; TOL, Tower of London; AC, anterior cingulate; VLFC, ventrolateral frontal cortex; MTC, medial temporal cortex (amygdala, hippocampus)
Preliminary studies suggest that behavioral/cognitive challenges may also be used to induce dopamine release in healthy subjects, therefore reflecting task-related regional dopamine release. In a landmark study, Koepp et al (1998) showed decreased $[^{11}C]$raclopride binding in the dorsal and ventral striatum during performance of a video game, which involved learning to navigate a tank through a battlefield for a financial incentive, compared to baseline. This associated striatal dopamine release with sensorimotor coordination, learning actions that predict reward and anticipating rewards, complementing previous findings seen in animals. Striatal dopamine release was also detected during planning of a set-shift on a modified card sorting task (again indexed by $[^{11}C]$raclopride binding potential reduction) (Monchi et al 2006a), indicating that dopamine transmission within the striatum increases during performance of specific executive processes, particularly those demanding cognitive flexibility. In addition to striatal dopamine release, studies have recently measured extrastriatal dopamine neuromodulation using cognitive tasks in healthy volunteers. Reduced $[^{11}C]$FLB 457 binding was observed in the ventrolateral frontal cortex, medial temporal cortex and ventral anterior cingulate during a working memory and sustained attention task (Aalto et al 2005), while decreased $[^{18}F]$fallypride binding in the thalamus was detected during a spatial attention task, and importantly, highly correlated with task performance (Christian et al 2006). These studies provide further evidence that extrastriatal dopamine transmission is critical for working memory and attentional processes.

Changes in tonic or baseline dopamine levels have also been estimated by increased $[^{11}C]$raclopride binding following AMPT in the striatum (e.g. Laruelle et al 1997a; Verhoeff et al 2001) and most recently after depletion of tyrosine and phenylalanine (TPD) (Mehta et al 2005) in healthy subjects. While increased $[^{11}C]$raclopride binding (indicating reduced tonic dopamine) was associated with worse sustained attention (Verhoeff et al 2001), this was not replicated in a subsequent study (Verhoeff et al 2003) and may have been confounded by detrimental effects of AMPT on mood and arousal. Using TPD, participants who became impaired in spatial working memory and planning accuracy were more likely to show greater reductions of tonic dopamine in the striatum (Mehta et al 2005). The latter findings suggest that changes in striatal dopamine levels can modulate certain cognitive processes, and that the level of cognitive impairment is dependent on the level of dopamine depletion. These PET studies measuring dopamine release and associations with cognitive performance are
summarized in Table 2-3. Nevertheless, while these studies are exciting and provide a novel means to further explore the dopaminergic basis of cognitive processes, such studies are still in their infancy and should be interpreted with caution due to technical and theoretical reasons relating to the imaging measurement itself (see Chapter 1).

2.5 SUMMARY

The above section reviewed a recent application of molecular imaging to examine the role of dopamine in a variety of human cognitive processes. Review of the literature suggests that PET and SPECT studies using various radioligands can be used to study the relationship between regional pre-, post- and intra-synaptic components of the dopamine system and human neuropsychological function, complementing previous studies in animals and behavioural studies in humans. The fact that correlations between dopamine indices and cognitive measures have been demonstrated across different diseases including PD, schizophrenia and Huntington’s disease but also healthy aging, provides greater strength to these relationships. Previous studies in animals and humans have demonstrated individual differences in the effects of dopamine on cognition, which may be due to basal levels of dopamine or baseline cortical efficiency (refer to introduction). An advantage of molecular imaging techniques is that individual differences in components of the dopamine system and therefore its modulation on cognition can be assessed.

A number of molecular imaging studies examining dopamine correlates of cognition have detected significant relationships within the striatum. Like those studies reviewed in Chapter 1, the striatum has been a particular focus partly because it is a large, high-density structure that is easily visualized and quantified with available dopamine radioligands. However, animal as well as functional imaging studies in humans suggest a critical role for cortical dopamine and D₁ receptors in the modulation of working memory and certain attentional processes. With the recent development of dopamine radioligands suitable for extrastriatal measurement (reviewed in Chapter 1), molecular imaging studies have provided preliminary support for these assertions, particularly in regard to PFC D₁ receptors and certain executive processes. Further exploration of the
extrastriatal dopamine system and its association with cognitive performance is clearly needed.

The above reviewed studies highlight the difficulty in referring to specific functions of brain structures, particularly the striatum, in isolation of the frontostriatal circuits. For instance, alteration of striatal dopamine seen in PD may not directly interfere with specific cognitive performance, but may do so indirectly, by disrupting the fronto-striato-thalamic loops. As such, associations between dopamine markers and cognitive measures need to be considered within the context of the fronto-striato-thalamic circuits.

Finally, a limitation of many of the reviewed molecular imaging studies concerns a lack of multiple comparison adjustment. This has occurred despite some studies performing multiple correlations across a number of regions or structures, and for a number of cognitive measures. Use of an appropriate multiple comparison procedure is important to minimize the probability of detecting a false statistically significant result when many tests are performed. As such, the control for multiplicity should be addressed in future studies, while keeping in mind the planned *apriori* study comparisons.
Chapter Three

3 Rationale of thesis

3.1 GENERAL THESIS OBJECTIVES AND AIMS

The goals of this thesis and PhD candidature were several-fold. The general goal of this PhD candidature was to learn the complexities of PET molecular imaging, as applied to the dopamine system, which was achieved through two independent PET studies. The primary objective of this thesis was to extend upon the literature assessing the various components of the dopamine system in the human brain, and evaluate these components in both striatal and extrastriatal regions. To this end, three radioligands with purported “extrastriatal” capabilities were assessed in either healthy or diseased brain, and changes in these radioligands were evaluated in consequence to dopaminergic alteration. Further, and in line with the increasing application of molecular imaging to investigate dopaminergic correlates of cognition, an exploratory component of this thesis was to investigate associations between dopamine markers and higher cognitive functions.

The general aims of this thesis were:

- To examine both striatal and extrastriatal dopamine transmission using PET
- To examine different components of the dopamine system (pre, post and intrasynaptic) using PET; Specifically,
  - To examine changes in “phasic” and “tonic” intra-synaptic dopamine transmission (Study 1)
  - To examine changes in pre- and post-synaptic dopamine transmission (Study 2), and
- To explore the relationship between regional PET dopamine markers and cognitive processes

3.2 SPECIFIC AIMS OF EXPERIMENTAL STUDIES

As mentioned, the current thesis conducted two independent PET imaging studies.
Study 1: This study was an investigative study that examined the sensitivity of the benzamide D2 antagonist radioligand, [18F]fallypride, to pharmacologically evoked changes in endogenous dopamine in healthy subjects. This study had three main aims: 1) to examine both amphetamine-induced and AMPT-induced changes in dopamine levels on [18F]fallypride binding in striatal and extrastriatal areas in the same subjects, 2) to examine the relationship between these pharmacologically-induced dopamine transmission modes and their relationship with cognitive function and 3) to examine the reproducibility of [18F]fallypride measurements within the brain. At the outset of this thesis the vulnerability of [18F]fallypride to dopamine manipulation had not been assessed in human subjects. Although this has recently been evaluated (Riccardi et al 2008; Riccardi et al 2006), Study 1 examined, for the first time, the sensitivity of [18F]fallypride to both amphetamine and AMPT challenges in the same subjects. This study has been published in the peer reviewed journal Synapse (see Appendix 2 for reprint).

Study 2: In contrast to Study 1, which focused on testing radioligand feasibility using a novel imaging paradigm, Study 2 focused on initial implementation of extrastriatal radioligands in clinical disease. Specifically, Study 2 examined changes in pre-synaptic and post-synaptic components of the dopamine system within the frontostriatal circuits in Parkinson Disease (PD), a disorder with known pathological alteration of dopamine. Pre-synaptic dopamine was assessed with [18F]FDOPA, a measure of dopamine synthesis and storage, whereas post-synaptic dopamine was assessed with [11C]NNC 112, a marker of post-synaptic D1 receptors. This study aimed to assess the relationship between pre-synaptic and postsynaptic dopamine markers within the frontostriatal circuitry and their relationship with executive processes in non-demented PD. It was hypothesized that PD patients, in comparison to healthy controls, would show reduced [18F]FDOPA uptake in the striatum and cortical areas and alteration of cortical [11C]NNC 112 binding. In addition, it was hypothesized that dopamine synthesis would negatively correlate with D1 receptor binding in cortex, and executive dysfunction in PD would correlate with [18F]FDOPA and [11C]NNC 112 alteration within the frontostriatal circuits. This study has been accepted for publication in the peer reviewed journal Psychiatry Research: Neuroimaging (see Appendix 3 for reprint of uncorrected proof). Results of this study were also presented as a poster at the “Dopamine 50 Years” symposium in Göteborg, Sweden, May 2007 (see Appendix 5 for copy).
3.3 RADIOLIGAND SELECTION

Three radioligands, \(^{18}\text{F}\)fallypride, \(^{18}\text{F}\)FDOPA and \(^{11}\text{C}\)NNC 112, were selected to evaluate intra-synaptic, pre- and post-synaptic components of the dopamine system. Primarily, these radioligands were selected over other PET and SPECT ligands because of their ability to assess their respective target in extrastriatal regions.

3.3.1 \(^{18}\text{F}\)Fallypride

As previously discussed in Chapter 1, several radioligands have recently been developed that measure extrastriatal D\(_2\) receptors, including \(^{11}\text{C}\)FLB 457, \(^{123}\text{I}\)epidepride and \(^{18}\text{F}\)fallypride. \(^{18}\text{F}\)Fallypride was selected over these other “extrastriatal” ligands because it can quantify D\(_2\) receptors in both the striatum and extrastriatal areas, whereas \(^{11}\text{C}\)FLB 457 and \(^{123}\text{I}\)epidepride are generally useful for D\(_2\) measurement in extrastriatal regions only (Christian et al 2000; Fujita et al 2000; Okubo et al 1999). Chapter 1 discussed the vulnerability of benzamide D\(_2\) receptor antagonist and agonist ligands to changes in endogenous dopamine levels. While D\(_2\) receptor agonists (e.g. \(^{11}\text{C}\)NPA and \(^{11}\text{C}\)MNPA) are more sensitive to endogenous dopamine competition (Narendran et al 2004; Seneca et al 2006), they cannot be used to evaluate extrastriatal dopamine transmission. The chemical structure of \(^{18}\text{F}\)fallypride is presented in Figure 3-1.

![Figure 3-1. Structure of \(^{18}\text{F}\)Fallypride](image)

3.3.2 \(^{18}\text{F}\)FDOPA

In addition to \(^{18}\text{F}\)FDOPA, pre-synaptic dopamine function can be assessed with various PET and SPECT ligands for the dopamine transporter. Dopamine transporter ligands are also reliably reduced in PD and may be a more sensitive indicator of dopamine neuronal loss than \(^{18}\text{F}\)FDOPA (Fujita and Innis 2002).
[\textsuperscript{18}F]FDOPA over DAT ligands was due to the thesis objective to measure pre-synaptic dopamine function in cortex. A number of PET studies have reportedly measured [\textsuperscript{18}F]FDOPA uptake in cortex, with both region and voxel-based methods (refer to Chapter 1). Due to the minimal presence of dopamine transporters outside the striatum, radioligands such as [\textsuperscript{18}F]FECNT or [\textsuperscript{123}I]β-CIT are not suitable for measuring cortical pre-synaptic dopamine function. In addition, [\textsuperscript{18}F]FDOPA is an old radioligand with established quantification methods and clinical utility in PD. The chemical structure of [\textsuperscript{18}F]FDOPA is presented in Figure 3-2.

![Figure 3-2. Structure of [\textsuperscript{18}F]FDOPA](image)

### 3.3.3 \textsuperscript{[11]C}NNC 112

The other common radioligand for measurement of post-synaptic D\textsubscript{1} receptors is the PET tracer \textsuperscript{[11]C}SCH 23390. \textsuperscript{[11]C}NNC 112 was selected over \textsuperscript{[11]C}SCH 23390 because it has greater specific to nonspecific binding ratios than \textsuperscript{[11]C}SCH 23390 (Halldin et al 1998), and thus more suitable for extrastriatal assessment of D\textsubscript{1} receptors. The chemical structure of \textsuperscript{[11]C}NNC 112 is presented in Figure 3-3.

![Figure 3-3. Structure of \textsuperscript{[11]C}NNC 112](image)
Chapter Four

4 General methods and principles

This chapter outlines the general methodology used in this thesis. Because this thesis evaluates PET technology as a molecular imaging tool to examine brain dopamine targets, the first section of this chapter will cover methodological aspects relating to PET imaging, including the principles and characteristics of positron emission detection, the basic criteria for PET radioligands and PET quantification. The following section introduces the methods used for assessing altered dopamine transmission in this thesis by means of pharmacological manipulations or use of a pathological sample. The last section will outline general methodology common to both experimental studies, including participant criteria, general scanning protocol and data analysis, partial volume correction and statistical analyses. Further description of the methodology specific to each experimental study will be detailed in their respective chapter (Chapter 5 and 6).

4.1 PRINCIPLES AND CHARACTERISTICS OF POSITRON EMISSION TOMOGRAPHY

4.1.1 Basis of PET imaging

Positron emission tomography is a nuclear medicine technique that enables the accurate and non-invasive detection of the in vivo concentration of radiopharmaceuticals labeled with positron emitters in the body or brain of living subjects. PET imaging is based on the principles of positron decay and coincidence detection of two gamma rays by radiation detectors (Cherry et al 2003). A typical PET scan involves many steps for successful completion – from isotope production in a cyclotron, chemical synthesis of the radiopharmaceutical, injection into the subject located within the PET gantry, to detection of gamma rays for subsequent image formation of the radiotracer concentration and distribution (Figure 4-1). Each of these steps is critically time dependent and requires the coordination and expertise of various disciplines.
In PET, short-lived positron-emitting radioactive isotopes, such as Carbon-11 ($^{11}$C), Nitrogen-13 ($^{13}$N), Oxygen-15 ($^{15}$O) or Fluorine-18 ($^{18}$F), are chemically incorporated into biological molecules (or analogs) normally used by the body, such as glucose, water or ammonia, or in the case of molecular imaging, into ligands for specific protein molecules (e.g. receptors, transporters or enzyme substrates). These positron-emitting radioisotopes each have their own characteristic properties such as half-life, positron energy and positron range (Table 4-1), which introduces some limits (albeit minimal) to the overall resolution and quality of the PET image (see later). The radiolabeled compound, otherwise known as a radiopharmaceutical, radiotracer or radioligand is intravenously injected into the peripheral circulation of a living subject, where it tags or “traces” the distribution of the molecule of biological interest. Upon injection, the unstable proton-rich radioisotope undergoes positron or positive beta decay, whereby it emits a positron (and neutrino) from the nucleus. The ejected positron will travel up to a few millimeters (depending on its kinetic energy and the density of tissue) whereupon it collides with an electron, resulting in pair annihilation of two 511 KeV gamma-ray photons in near 180-degree opposing directions (Figure 4-2a). These photon pairs are detected with multiple rings of scintillation crystals surrounding the subject’s head that are coupled to photomultiplier tubes to convert the light photons to a measurable electrical pulse. Pair annihilation of the 511 KeV photons is detected as a “true” event if detected in coincidence or within a narrow time window of several nanoseconds (Figure 4-1).
4-2b). The spatial location of the line of coincidence of the two gamma rays is recorded, thus making it possible to localise the source of the annihilation event along the straight line of projection (or response). The data of thousands of such coincident lines are collected at different view angles in multiple frames. These projections are corrected for factors such as attenuation, scatter and random coincidences before being reconstructed to form an image of the radioactivity distributed over time in the subject’s brain (Bailey et al 2005a; Cherry et al 2003; Mullani and Volkow 1992; Saha 2005). Therefore, these regional levels of radioactivity are assumed to reflect the distribution and concentration of the underlying biological process (e.g. receptor density, glucose metabolism or blood flow) that the radiolabeled compound is deemed to measure.

**Table 4-1. Properties of some positron-emitting radioisotopes**

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Half-life (min)</th>
<th>Max. energy (MeV)</th>
<th>Positron range (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen-15</td>
<td>2.03</td>
<td>1.72</td>
<td>2.4</td>
</tr>
<tr>
<td>Nitrogen-13</td>
<td>9.96</td>
<td>1.19</td>
<td>1.3</td>
</tr>
<tr>
<td>Carbon-11</td>
<td>20.4</td>
<td>0.96</td>
<td>0.9</td>
</tr>
<tr>
<td>Flourine-18</td>
<td>109.8</td>
<td>0.635</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Table adapted from Bailey et al (2005a) and Mullani and Volkow (1992)

Because of its exceptional sensitivity (see below), PET can create high-quality images with only nanomolar to picomolar concentrations of compounds, allowing trace amounts of compounds or drugs to be administered and followed over time without perturbing the biological system. Therefore, radioligands administered at tracer doses produce no pharmacological effects, label less than 1% of receptors or target proteins, and are disposed like all drugs in respect to metabolism and distribution. In the current thesis, $^{18}$F and $^{11}$C radioisotopes were used to label the dopamine synthesis enzyme, AADC ($[^{18}$F]FDOPA) and dopamine D$_1$ ($[^{11}$C]NNC 112) and D$_2$ ($[^{18}$F]fallypride) receptors. Trace amounts of these radioligands ensure that the respective pre- and post-synaptic components of the dopamine system are not perturbed.
Figure 4-2. Positron detection with PET. a) positron-electron annihilation produces two antiparallel 511 KeV photons. b) localisation of the annihilation event is determined by coincidence detection of the two photons by two of the detectors positioned around the subject’s head; reconstruction of thousands of coincidence pairs subsequently results in an image of the isotope distribution.
4.1.2 Spatial, temporal and sensitivity parameters of PET technology

Neuroimaging techniques with radionuclide technology (e.g. PET and SPECT) and nuclear magnetic resonance technology (e.g. MRI) are commonly characterised by the ability with which they can distinguish detail on spatial, temporal but also sensitivity scales. Spatial resolution refers to the ability to distinguish two separate objects positioned in close spatial proximity, whereas temporal resolution refers to the ability to detect events occurring in close temporal proximity (Mazziotta 1996). In contrast, sensitivity refers to the lowest in vivo concentration of the target measurement that can be clearly distinguished from background. Table 4-2 summarises the spatial and temporal resolution and sensitivity of PET and MR imaging techniques.

<table>
<thead>
<tr>
<th>Imaging Technique</th>
<th>PET</th>
<th>MRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spatial Resolution</td>
<td>2 – 6 mm FWHM</td>
<td>&lt; 1 mm FWHM</td>
</tr>
<tr>
<td>Temporal Resolution</td>
<td>Minutes</td>
<td>Seconds</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>~ 10^{-12} M</td>
<td>~ 10^{-4} M</td>
</tr>
</tbody>
</table>

FWHM, Full Width Half Maximum; refers to the distance at which two separate foci may be distinguished. Table compiled from Fujita and Innis (2002).

From inspection of Table 4-2, it is apparent that the major advantage of PET lies in its superior sensitivity (~10^{-12} M). In PET, sensitivity relates to the detection of radioactivity (or specifically the “true” coincidence events) by the system (Mullani and Volkow 1992). PET radiation detectors can easily measure minute amounts of the radioisotope, typically in the nanomolar to picomolar concentration range. This extraordinarily high sensitivity is several orders of magnitude greater than the sensitivity of MRI, which generally limits biological measurements to the millimolar range (see Table 4-2) (Cherry et al 2003; Fujita and Innis 2002). Because molecules pertaining to the dopamine system are present in concentrations of less than 10^{-8} M, PET is currently the only available technique capable of quantifying dopaminergic receptors, transporters and enzymes, in vivo, in human brain. Therefore, the high
sensitivity of PET for the radioisotope (e.g. $^{18}$F and $^{11}$C), along with the use of selective compounds (i.e. FDOPA, fallypride and NNC 112; see Chapter 1 and 3), make it possible for the current thesis to examine specific pre- and post-synaptic dopamine components in even low density regions of living brain.

In contrast to its superior sensitivity, PET has limited anatomic resolution and poor temporal resolution (Table 4-2). In comparison with MRI, the kinetics of PET tracers occur over a slow time course (of minutes), and therefore cannot temporally track, for instance, fast changes in chemical concentration or neuronal activity that they may be intended to measure. Likewise, the spatial resolution of a PET image is lower than that provided by MRI, although improvements in scanner instrumentation (relating to crystal and electronic signaling characteristics) and three-dimensional (3D) acquisition and reconstruction strategies have greatly enhanced resolution, as well as sensitivity (see Bendriem and Townsend 1998; Boellaard et al 2004). Currently, anatomic resolution of 2 – 2.5 mm is provided with dedicated high resolution research tomograph devices (de Jong et al 2007), although these are still in their relative infancy and are not widely used for human brain imaging studies. In addition to the physical properties of the scanners, spatial resolution of the camera is affected by the intrinsic properties of the radioisotope and annihilation event. For instance, the distance between the annihilation event and the emitting nucleus (i.e. positron range), is determined by the positron energy (see Table 4-1), and introduces some uncertainty to the localization of the decaying nucleus. Further, the small angular deviation of the two annihilated photons from 180° contributes to a slight misalignment of the photon trajectory. Therefore, both positron range and acollinearity of the annihilated photons sets an absolute limit on the spatial resolution attainable with PET technology.

4.1.3 Positron detection and system performance: effects on image quality and quantitation

Photon characteristics

The quality and accuracy of the radioactivity measurement by PET is complexly affected by the characteristics of positron detection and photon statistics as well as the performance characteristics of the PET instrumentation. For instance, the interaction of
annihilation photons with surrounding tissues, as well as the nature of electronic coincidence detection contributes some error in image quantitation, namely due to the misregistration of valid coincidence events or reduced photon detection by the PET camera. These effects include photon attenuation, scatter and random coincidences. Attenuation of photons occurs when one or both of the annihilated photons undergoes total absorption in tissue, which consequently reduces the total number of photons reaching the detectors and thus reduces the statistical quality of the image. In contrast, scattered radiation arises when one or both of the emitted photons deviate from its original path, due to the photon interacting with tissue, or due to photon deflection within the detectors or off camera components. Scattered events (i.e. photon scatter that is detected within the coincidence time window and registered as a true event) introduces a source of error in positron localization as it leads to misalignment of the lines-of-response, causing blurring of the image and poor image contrast. A further, direct consequence of the coincidence timing window used for positron detection is the acquisition of random events by the PET scanner. Random events occur due to the accidental detection of two unrelated events within the pre-defined coincidence timing interval, producing a false coincidence. Random events will again add error in positron localization, providing misaligned or erroneous lines of response, and will add uncorrelated background counts to the image. Having a narrow coincidence window will assist in reducing the occurrence of random events; however, this may result in a trade-off with the number of true counts detected. While photon attenuation, scatter and randoms represent a source of error in PET images, these effects can be partially corrected using various techniques and algorithms prior to image reconstruction (Bailey et al 2005a; Mullani and Volkow 1992; Porenta 1994).

**Photon statistics**

A further determinant of overall image quality relates to photon statistics - that is, the number of photons detected by the PET camera. Photon attenuation, the electronic “dead-time” interval following detection of an event, and the count-rate capability (i.e. speed of data collection without saturation) of the camera are factors that can affect overall system sensitivity and the statistical quality of the emission data. In addition to these factors, the scanning period will also affect photon count rates, with longer scanning times increasing the number of total counts detected (Mullani and Volkow
Spatial resolution, partial volume effects and quantitation

The spatial resolution of the scanner is an important determinant in the precision of quantitative brain studies, as the resolution of an image directly affects the ability to differentiate between two small, closely spaced objects. As a general guideline, object size must be at least two times the full-width at half maximum (FWHM, a measure of resolution) of the instrument to accurately recover its true activity (Mullani and Volkow 1992). In brain imaging studies, this factor becomes particularly pertinent, as many brain structures of interest are smaller than the FWHM of the instruments, particularly of early instruments used in initial PET studies. Limited spatial resolution results in alteration of the true count rate between different regions, a phenomenon known as the partial volume effect (Meikle and Badawi 2005; Mullani and Volkow 1992). Partial volume effects refer to the loss of signal of structures smaller than two times the FWHM of the tomograph, as well as to the contamination from adjacent brain tissue or regions (Muller-Gartner et al 1992; Rousset et al 1998; Saha 2005). These effects can result in underestimation of activities to small structures due to dilution or “blurring” of radioactivity from the region of interest to the background, or due to averaging of activities from surrounding tissue (such as white matter, bone or cerebrospinal fluid) (Muller-Gartner et al 1992; Porenta 1994). These alterations of true regional concentration therefore impact the ability to accurately quantify small regions in the neocortex and subcortex of the brain as well as brainstem nuclei.

The effects of partial volumes on the reconstructed image can be partially corrected with several algorithms or mathematical approaches (see below). Likewise, partial volume effects can be minimized by accurate delineation of the regions of interest (ROI), e.g. by using anatomical MRI to identify regions after coregistration of PET and MRI data. As discussed in Chapter 1, the poor resolution of PET tomographs and resulting partial volume effects restricted early dopamine imaging studies to large brain structures such as the striatum. Recently however, smaller structures have been able to be quantified, namely due to the increased spatial resolution and sensitivity of the PET instrumentation, and to the development of the aforementioned imaging techniques to correct or minimize any partial volume effects (see below for a description of the partial
volume correction used in the current thesis). As outlined in Chapter 1, a number of recent studies have thus examined smaller structures in the neocortex or have delineated the striatum into its functional subdivisions. This thesis will extend upon these studies by also quantifying striatal subdivisions and small regions outside the striatum.

**Instrumentation and system characteristics**

The precision of image quantitation and quality is not only affected by the characteristics of the photons but by the attributes and performance of the PET system itself. These factors, however, are interrelated, as the camera and system specifications will directly impact for instance, the extent of scatter, randoms and radiation count rates, some of which have been discussed above. Performance measurements of PET instrumentation include spatial and temporal resolution and sensitivity as well as count-rate efficiency and electronics dead-time characteristics. Although beyond the scope of the current chapter, the specific design of the PET system, such as the type of crystal, number of rings, the size, number, geometry and configuration of the detectors, electronic circuits as well as the correction and reconstruction methods will determine these performance measurements, which will in turn affect the quality of the PET image (Mullani and Volkow 1992). Scanner designs and specifications not only vary between systems but continuously evolve to improve performance. In particular, systems have focused on achieving even higher sensitivity, as higher sensitivity, in general, is associated with better signal-to-noise of the reconstructed image (Bailey 2005). Likewise, spatial resolution has also undergone significant improvement in recent years due to scanner development, particularly with the use of 3D PET, smaller detectors and faster crystals and electronics. Further information about the effects of PET instrumentation on image acquisition and quantitation is provided by Bailey (2005), Mullani and Volkow (1992), and (Porenta 1994). In addition, a comprehensive background to PET technology and nuclear medicine is provided in the following reference books, Bailey et al (2005b), Cherry et al (2003), Saha (2005), and Wernick and Aarsvold (2004).

### 4.2 OVERVIEW OF PET RADIOLIGAND DEVELOPMENT AND CRITERIA

Molecular imaging with PET and SPECT relies on the use of radiolabeled compounds
suitable for studying \textit{in vivo} biochemical processes in the brain. In contrast to ligands used for \textit{in vitro} imaging, or for \textit{in vivo} imaging of the body, there are specific and relatively more stringent requirements for \textit{in vivo} brain imaging probes due to obstacles posed by the blood-brain barrier (BBB) as well as the brain itself. Provided below is a brief description of the general steps involved in radioligand development and the properties required of a ligand for successful \textit{in vivo} imaging of molecular targets.

The development and evaluation of potential new ligands involves several \textit{in vitro} and \textit{in vivo} steps. \textit{In vitro} studies characterize several properties of the ligand, including its affinity and selectivity for the molecular target, the concentration of binding sites (B\textsubscript{max}) as well as its reversibility (Kegeles and Mann 1997). The optimal affinity (i.e. dissociation equilibrium constant) of the ligand should preferably be lower than the B\textsubscript{max} of the target sites; e.g., with a nonomolar concentration of binding sites the ligand affinity should ideally be in the subnanomolar range (Halldin et al 2001). Radioligand affinity should be relatively high, in order to achieve a high ratio of target-to-non target (i.e. specific-to-nonspecific) uptake in the brain – a factor important for obtaining reliable data and for detecting small concentrations of target sites, or small changes in target site availability due to disease or drug occupancy (Fujita and Innis 2002; Halldin et al 2001). This is particularly important in relation to the experimental studies of the current thesis as possible changes in dopamine radioligand parameters caused by both pharmacological manipulation (Study 1; chapter 5) and disease (Study 2; chapter 6) will be assessed. For extrastriatal measurement of molecular targets radioligands need to display even higher affinity and specific-to-nonspecific ratios than that required for high-density regions such as the striatum. Further, in order to examine specific molecular targets (such as D\textsubscript{2} receptors), ligands should have high selectivity (i.e. highest binding affinity) for the target site of interest relative to non-target sites. This includes displaying high selectivity over other neurotransmitter systems, such as selectivity for the dopamine over the serotonin system, as well as within the distinct receptor family (e.g. D\textsubscript{1}- and D\textsubscript{2}- like) and receptor subtypes (e.g. D\textsubscript{2}, D\textsubscript{3}, D\textsubscript{4}). In practice however, dopaminergic ligands do not show pure selectivity for distinct subtypes of the same receptor family. Further, estimation of the non-saturable component and reversibility of the ligand are also important considerations of candidate agents, relating to the level of non-specific binding of the ligand and its kinetic suitability. Although these parameters are difficult to estimate \textit{in vivo}, they may be...
related to the degree of lipophilicity and affinity of the ligand. Non-specific binding generally reflects binding to proteins and lipids, increases with increasing lipophilicity and contributes to the background activity of the image. In contrast, the reversibility of the ligand is in part determined by its binding affinity, as higher affinity contributes to a greater rate of association (or “stickiness”) to dissociation from the binding site (Fujita and Innis 2002). Ligand reversibility will influence whether reversible or irreversible models are required for image quantification (see following section). For reversible interactions, clearance or washout of the ligand must be fast enough to occur within the time frame of the imaging measurement allowed by the half-life of the radioisotope (Fujita and Innis 2002; Halldin et al 2001).

As mentioned above, the development of radioligands for in vivo brain imaging is challenging due to obstacles posed by the brain itself. In particular, this relates to the specific but contrasting combinations of molecular weight, affinity and lipophilicity of the ligand for adequate uptake across the BBB and for adequate ratios of specific-to-nonspecific binding and kinetic characteristics (Fujita and Innis 2002). Clearly, a ligand with a small molecular weight and moderate to high lipophilicity is required for adequate brain penetration and uptake, which is essential for obtaining reasonable counting statistics. However, in the brain the situation becomes more complex, with high ligand affinity but low lipophilicity needed for achieving high levels of specific, but low non-specific binding, respectively. As discussed above, although high affinity is a prerequisite for measurement of low-density regions of the molecular target, it shouldn’t be so high that the tracer shows negligible clearance from the tissue which limits its use for quantification. High lipophilicity, in contrast, will increase non-specific binding to proteins and lipids, and will also increase binding to plasma proteins, reducing the availability of the free ligand to cross the BBB (Fujita and Innis 2002; Halldin et al 2001). This optimal, but very narrow window of affinity and particularly lipophilicity of brain radioligands is arguably a major factor in the paucity of good agents for in vivo brain imaging.

Following initial in vitro characterization of potential candidate ligands, in vivo experiments, typically using pretreatment or displacement protocols, are performed in animals such as rodents and non-human primates to further characterize and confirm the ligand properties in vivo. Some of these parameters have been discussed above and
include; extent of brain permeability or brain penetration, the specificity, distribution, clearance and metabolism of the tracer as well as its vulnerability to competition with the endogenous ligand. Some of these studies can be complemented with receptor autoradiography techniques, particularly for providing information regarding ligand distribution and non-specific binding (Halldin et al 2001; Kegeles and Mann 1997). Metabolite characterization is particularly important, as PET cannot distinguish the chemical form of the radioligand. If a radiometabolite enters the brain, it will either falsely increase specific binding (if the metabolite binds to the receptor) or increase nonspecific binding (if the metabolite has negligible affinity for the receptor). Thus, the radiometabolites of the radioligand should not enter the brain. Finally, before its extended use in humans, radioligands must display a safe radiation-absorbed dose profile based on human dosimetry and biodistribution studies and safe toxicological properties (Kegeles and Mann 1997). Approval for human use is typically obtained through the Food and Drug Administration (or applicable body outside the United States) via a research Investigational New Drug application.

4.3 KINETIC MODELING AND QUANTIFICATION OF PET RADIOLIGANDS: REFERENCE TISSUE MODELS

As described in the preceding sections, PET allows measurement of time-related regional activity concentrations of radiolabeled compounds specifically developed to measure one or more physiological parameters of interest, such as receptor count, biochemical reactions, blood flow and metabolism. PET and SPECT ultimately aim to measure these physiological parameters, but the actual experimental data are the measured radioactivity accumulation during image acquisition. Ideally the uptake and distribution of a radiotracer (or ligand) would only be determined by the properties of the biological parameter – be that receptor concentration, synthesis or metabolic activity that governs the interaction between the ligand and the physiological system. In reality however, there are many other variables that can influence the in vivo behaviour of the tracer, including injected dose, regional blood flow, capillary permeability, plasma protein binding, non-specific binding and tracer metabolism, all of which can contribute to the observed activity image obtained by PET. In order to control or minimize these extraneous factors and relate the measured radioactivity to the desired physiological properties under study, mathematical modeling of the PET data is required. Without
such modeling, the contribution of other unrelated factors to the resultant PET image cannot be determined – an issue particularly important when examining the effects of drugs (which can have peripheral effects) or disease on target molecules, both of which are being examined in the current thesis (Carson 2005; Kegeles and Mann 1997).

There are a variety of modeling approaches to quantify molecular targets with radioligand imaging, with the choice of model depending on the characteristics of the specific ligand, the ligand infusion method (e.g. bolus or bolus and constant-infusion), as well as the model’s own computational and technical demands. However, regardless of the approach, all mathematical models, with knowledge of the underlying physiology, describe by use of equations or “rate constants” the movement or kinetic properties of the particular tracer in the biological system. In essence, the goal of modeling (otherwise known as tracer kinetic modeling) is to estimate, by fitting the model to the experimentally obtained tissue radioactivity measurements, these kinetic parameters or rate constants. These rate constants subsequently derive a quantitative measure of a particular physiological process (e.g. binding, delivery, uptake etc) that is distinct from all other processes contributing to the PET signal (Carson 2005; Morris et al 2004). Models can be fitted to time activity data within individual tissue regions or organs or to each image pixel to yield a functional parametric map of the tissue outcome measure. Because of the limited information provided by PET, models are simplifications of the real behaviour of a tracer molecule in a system, and thus application of a simple model to a complex system relies on certain assumptions and conditions to be satisfied (Carson 2005). In molecular imaging, the most common methods for ligand quantification (using a bolus injection) are with “compartmental” or “graphical” models, both of which can be applied to reversibly and irreversibly bound tracers, and both of which are used in the current thesis.

4.3.1 Reversible versus irreversible ligands

Movement of a tracer throughout the biological system following tracer injection can follow various paths. In simple descriptions, this includes arterial delivery of the tracer to the tissue of interest, its interaction with the tissue, and venous outflow of the tracer or its metabolites back to blood. The type of interaction with tissue sites may be
reversible, whereby dissociation from the binding site occurs, or irreversible, whereby the tracer is “trapped” in the tissue of interest, undergoes a biochemical reaction or dissociates from the binding site well beyond the image acquisition. Whether the ligand behaves in a reversible or irreversible manner during the time frame of the PET acquisition will determine the modeling approach to be used. For reversible ligands, the kinetics are typically characterized by the rate of tracer uptake and washout over time, generating a time-activity curve of regional radioactivity. Reversible radioligands typically approach equilibrium during the time period of the experiment, thus allowing transient equilibrium methods to be used. In contrast, graphical analysis of irreversible ligands is typically based on a component of the uptake curve for conversion to a straight-line plot.

In the current thesis, both reversible and irreversible radioligands were used to quantify dopamine components with use of different modeling approaches. Both dopamine receptor radioligands, $[^{11}\text{C}]$NNC 112 and $[^{18}\text{F}]$fallypride bind reversibly with their respective receptor and allow analysis of the uptake and clearance components of the time-activity curve. $[^{18}\text{F}]$FDOPA is an irreversible tracer, as the desired physiological process is the unidirectional uptake of $[^{18}\text{F}]$FDOPA, which is converted to $[^{18}\text{F}]$fluorodopamine in tissue. For $[^{18}\text{F}]$FDOPA, a graphical analysis suitable for irreversible tracer quantification is used.

### 4.3.2 Compartmental versus graphical models

#### Compartmental models

The most commonly used model for describing the uptake and clearance of radioactivity tracers in tissue is compartmental analysis. According to this model, a tracer molecule at any given time will exist in one of several compartments, which are defined as a space in which the tracer concentration is uniform. The compartmental model describes the exchange or movement of the tracer between these specified compartments via first-order kinetics or rate constants. In other words, these rate constants define the fractional rate of change in tracer concentration between compartments per unit of time. The physiological meaning of these rate constants depend on the “states” defined by each compartment, which are then related to the properties or processes under study (Carson...
2005; Kegeles and Mann 1997).

In receptor imaging studies, the concentration and movement of radioligands are usually described with a three compartmental model (Figure 4-3). The first compartment is the arterial blood or plasma. From this compartment the radioligand passes through the BBB into the non-displaceable compartment which consists of free and non-specifically bound radioligand. For these radioligand states to be considered as one compartment, the free and non-specifically bound radioactivity must be in rapid equilibrium with each other. The third compartment is the region of specifically bound ligand, consisting of high-affinity receptors. The rate constants $K_1$ and $k_2$ define the delivery and clearance of the radioligand across the blood-brain barrier. The rate constants between the non-displaceable and specific binding compartments represent the movement of radioligand on ($k_3$) and off ($k_4$) the receptor (Carson 2005; Ichise et al 2001).

The most common outcome measure derived from compartmental modeling of reversibly bound receptor radioligands is the binding potential ($BP$), which equals (or reflects) the ratio of receptor density ($B_{\text{max}}$) to the radioligand equilibrium dissociation constant ($K_D$) (Innis et al 2007; Mintun et al 1984). In in vivo imaging, binding potential reflects the ratio at equilibrium of specific binding concentration to some reference concentration, such as radioligand in free plasma, total plasma or non-displaceable uptake (Innis et al 2007). These values are obtained by measuring the ratio of various kinetic rate constants of a three compartmental (two-tissue) model to reflect the true in vitro measurement of $B_{\text{max}}/K_D$. Over the years, different nomenclature have been used to denote the binding potential outcome measure (which will not be reviewed here),
generally because of the use of the three different reference concentrations outlined above. Recently, consensus nomenclature has been reached to describe in vivo binding potential values obtained by these three reference concentrations, as defined by comparison of specific binding to free plasma (\(BP_F\)), total plasma (\(BP_P\)) and non-displaceable uptake (\(BP_{ND}\)) (as used in the current thesis but refer to section 4.3.3) (Innis et al 2007). The current thesis will adopt this consensus nomenclature for denoting binding potential measurements for \([^{11}C]NNC 112\) and \([^{18}F]fallypride\).

For compartmental analysis, several assumptions are typically made to reduce the number of kinetic parameters. Key assumptions include; that the radioligand in the system comes from a single source, i.e. the arterial plasma; that each compartment is well-mixed, such that no concentration gradients exist; that the rate constants describing the system do not change over time during the study; that first-order kinetics can describe the transfer of radioligand between compartments and that the radioligand non-specifically bound equilibrates rapidly with free tissue radioligand (Carson 2005; Ichise et al 2001).

**Graphical models**

Graphical analysis is another commonly employed method for quantification of tracer kinetic data. In short, this general linear method involves graphing the measured time activity data against other measurable data, resulting in a straight-line plot whose slope and intercept has physiological meaning (Carson 2005). Although the graphical approach typically involves further simplifications of the true physiology than compartmental models, it is generally less computationally demanding with fewer parameters (slope and intercept) to estimate, is simple to confirm the linearity of the data, and can be easily applied on a pixel-wise basis to generate a parametric map of each parameter.

Graphical analysis methods can be applied to both reversibly and irreversibly binding tracers using an input function provided from plasma or reference region (see section 4.3.3) measurements (Logan 2000; Logan 2003). The Patlak plot (Patlak and Blasberg 1985; Patlak et al 1983) is the most commonly used graphical analysis technique for tracers displaying irreversible trapping in the system, such as \([^{18}F]fluorodeoxyglucose\).
or \[^{18}\text{F}]\text{FDOPA}. In brief, the measured data undergoes a mathematical transformation and is plotted against a “normalized time,” resulting in a straight line for systems with irreversible compartments and the derivation of an uptake constant from the slope of the plot (see section 4.3.4 for further detail) (Carson 2005; Patlak et al 1983). For reversibly binding receptor radioligands, the most common graphical approach is the Logan graphical analysis (Logan et al 1990), which, after transformation of the acquired PET data, estimates the total volume of distribution (i.e. the ratio of the tissue concentration to blood concentration at equilibrium) from the slope of the linear plot. Graphical analysis of tracer kinetic data will be utilized in the current thesis.

### 4.3.3 Use of reference tissue in PET quantification: Reference tissue models

As mentioned above, tracer kinetic modeling is based upon the relationship between the measured PET data and the input function. Typically, activity measurements made from the blood is used to define the input function into the system (e.g. the first brain tissue compartment). While the use of an arterial input allows more direct estimates of model parameters to be made and generally involves fewer assumptions, arterial sampling is an invasive procedure often causing discomfort to the subject and is technically demanding. For example, arterial sampling requires measurement and correction for radioactive metabolites for determination of the parent radioligand in plasma, can often involve correction for the fraction of the radioligand that is bound to plasma proteins (i.e. measurement of the free ligand), and involves a different coincidence detection to that of the PET scanner which may lead to slight error in activity measurements (Ichise et al 2001; Innis et al 2007). Because of these disadvantages associated with blood sampling, methods have been developed to avoid the measurement of the arterial input function altogether, while still providing parameter estimation by kinetic modeling. These methods, called reference models, replace the plasma input curve with an indirect input curve, that is, the time-activity curve of some reference tissue devoid of the target or specific binding sites. To provide an example with compartmental configuration, reference tissue represents the non-displaceable tracer compartment and does not have the specific binding compartment. These methods compare the time-activity curve in a region of interest to that in a reference region and, assuming certain relations between the kinetics of both regions, kinetic parameters (typically simplifications or ratios of the
original parameters) can be identified (Carson 2005; Morris et al 2004). Reference models rely on additional key assumptions, including that the reference region shows negligible specific binding sites (e.g. of receptors or enzymes), and that the nondisplaceable uptake is the same among regions of brain and does not differ among subject groups or treatment effects (Innis et al 2007; Morris et al 2004).

Reference tissue models are commonly applied to receptor imaging studies by comparing radioligand concentration in receptor-rich and receptor-free regions. Various models have been developed to derive estimates of binding potential, defined as $BP_{ND}$, which denotes the ratio at equilibrium of the concentration of specifically-bound radioligand to that of nondisplaceable uptake (Innis et al 2007). Graphical methods have also made use of the reference region approach for both reversible (e.g. the Logan noninvasive plot) and irreversible (e.g. the Patlak plot) ligands (Logan et al 1996; Patlak and Blasberg 1985).

### 4.3.4 Reference tissue models used in the current thesis

Several reference region models were employed in the current thesis to quantify the three dopamine radioligands, $[^{18}F]$fallypride (Study 1), $[^{11}C]$NNC 112 and $[^{18}F]$FDOPA (Study 2). Various compartmental and graphical analysis techniques suitable for both reversible and irreversible ligands were used. Compartmental models, with the cerebellum as the reference region, were applied to $[^{18}F]$fallypride and $[^{11}C]$NNC 112 PET measurements for estimation of dopamine D$_2$ and D$_1$ receptor concentrations ($BP_{ND}$), respectively. A graphical method, using the occipital cortex as the reference region, was applied to $[^{18}F]$FDOPA data for the measurement of irreversible $[^{18}F]$FDOPA uptake into the tissues of interest (AADC-rich). The three methods are briefly described below. For further detail please refer to the cited references for each model and to each experimental chapter of this thesis.

**Simplified reference tissue model (SRTM)**

The SRTM of Lammertsma and Hume (1996) was used to estimate regional $[^{18}F]$fallypride $BP_{ND}$. This model is a simplification of the four parameter reference tissue model (Lammertsma et al 1996), which reduces the number of fit parameters.
from four to three. Unlike most receptor parameter models, the SRTM is based on a one-tissue compartmental configuration to decrease the number of parameters. The model assumes that the radioligand time-activity curve in both the reference and the target regions can be fitted by this one-tissue compartmental configuration. The SRTM consists of three unknown parameters: \( R_1 \) (relative ligand delivery, defined as \( K_1/K'_1 \)); \( k_2 \); and the parameter of interest, \( BP_{ND} \), which are fitted using nonlinear regression techniques (Lammertsma and Hume 1996). The SRTM was chosen because it has previously been used in \([^{18}F]Fallypride\) studies (Riccardi et al 2006; 2008) and because it provided the best fit of \([^{18}F]Fallypride\) data.

**Multilinear reference tissue model 2 (MRTM2)**

Ichise’s MRTM2 model (Ichise et al 2003) was applied to \([^{11}C]NNC 112\) PET measurements to derive regional \( BP_{ND} \). The MRTM2 model is a modification and simplification of the original MRTM and is resistant to noise and computationally fast for generation of parametric images. The model involves estimation of \( k'_2 \), the clearance rate constant from the reference region, and then fixing \( k'_2 \) for pixel-wise calculations, reducing the number of fitted parameters from three to two (Ichise et al 2003). The two outcome parameters for MRTM2 are \( R_1 \) (\( K_1/K'_1 \)) and the outcome measure of interest \( BP_{ND} \). Because \( ^{11}C \) has a shorter half-life than \( ^{18}F \) (20 minutes compared to 110 minutes), adding comparatively more “noise” to the data, the MRTM2 was used over SRTM as it is more resistant to noise.

**Patlak plot**

As mentioned above, irreversible uptake of \([^{18}F]FDOPA\) was calculated with the Patlak graphical analysis using a reference region approach (Patlak and Blasberg 1985). Application of a reference region method can be employed when the tissue does not irreversibly trap the tracer. In the current thesis, the \([^{18}F]FDOPA\) time-activity curve in the occipital cortex served as the reference tissue to replace plasma activity as the input function. The Patlak plot assumes reversible transfer of the radioligand across the blood-brain barrier but irreversible trapping of some of the radioligand in areas with specific signal. In contrast, the reference region has only reversible transfer of the radioligand across the blood-brain barrier (Patlak and Blasberg 1985; Patlak et al 1983). Conceptually, the irreversible tissue region can be illustrated by a compartmental model.
in which the rate constant, \( k_4 \), is zero, indicating irreversible tracer accumulation. In the Patlak method, the ratio of the measured tissue to input function activity is plotted against a “normalized time” (a distortion of time based on the shape of the input function), resulting in a linear plot after appropriate equilibration time and the derivation of a linear equation. The outcome measure for the Patlak plot is the slope of the linear regression, which equals \( K_i \) and represents the influx rate constant of the tracer into the irreversible compartment of the system (Carson 2005; Patlak and Blasberg 1985; Patlak et al 1983). The Patlak model was used in this thesis as it has been applied and validated in numerous \(^{18}\text{F}\)FDOPA studies, including recent studies measuring \(^{18}\text{F}\)FDOPA uptake in cortex.

4.4 METHODS FOR ASSESSING ALTERED DOPAMINE TRANSMISSION

Both pharmacological and pathological methods for compromising the human dopamine system were used in this thesis. Pharmacological challenges consisted of: 1) acute stimulation of dopamine release with \( d \)-amphetamine and 2) transient dopamine depletion with AMPT (Study 1). The “pathological” method involved studying a patient population (Parkinson disease) with a known deficiency in dopamine transmission (Study 2).

4.4.1 Pharmacological stimulation of dopamine release with \( d \)-amphetamine

In Study 1 of the current thesis, \( d \)-amphetamine (otherwise known as amphetamine) was administered to pharmacologically increase extracellular levels of dopamine within the striatum and extrastriatal regions of the brain. Amphetamine acts on the pre-synaptic dopamine neuron via two main mechanisms to induce a large and transient increase in dopamine into the intra-synaptic space. First, amphetamine redistributes dopamine from the vesicular to the cytoplasmic pool and second, it promotes the release of dopamine by reverse transport from the cytoplasmic pool to the synapse through the dopamine transporter (Fischer and Cho 1979; Sulzer et al 1995). Amphetamine-induced dopamine release is reliant on new dopamine synthesis, vesicular transport mechanisms as well as DAT. As such, amphetamine-induced dopamine response can be blunted by inhibition of dopamine synthesis with AMPT, inhibition of the vesicular monoamine transporter
via reserpine and blocking the dopamine transporter via dopamine reuptake inhibitors (Laruelle 2000). Likewise, these agents (AMPT, reserpine and the dopamine transporter blocker GBR 12909) also attenuate the effects of amphetamine on radioligand binding potential (Innis et al 1992; Laruelle et al 1997b; Villemagne et al 1999), providing evidence that changes in amphetamine-induced radioligand binding reflect changes in synaptic dopamine levels (see Laruelle 2000), an assumption important in the interpretation of Study 1 in the current thesis.

The efflux of dopamine from amphetamine is large in magnitude but relatively short-lived, mimicking the high-amplitude, transient release of dopamine from burst firing or “phasic” modes (see Chapter 1). Microdialysis studies in non-human primates show that 0.2 – 0.5 mg/kg i.v. amphetamine increases extracellular dopamine by about 450% – 1400% above baseline values (Breier et al 1997; Laruelle et al 1997b). Extracellular dopamine increases rapidly, peaking within 10 – 20 min after amphetamine injection followed by a rapid decline thereafter (Laruelle et al 1997b). Although percent reductions of $[^{11}\text{C}]$raclopride or $[^{123}\text{I}]$IBZM binding potential are substantially smaller (with decrements of about 10 – 28% of baseline values) than the percent increases in extracellular dopamine, these measurements are linearly correlated, demonstrating that large increases in extracellular dopamine release is associated with relatively small decreases of radioligand binding (Breier et al 1997; Laruelle et al 1997b). This linear relationship between microdialysis and imaging measurements again provide support for the $\text{in vivo}$ binding competition model, indicating that changes in radioligand binding are dependent on changes in synaptic levels of dopamine (Laruelle 2000), despite being a “low-gain” monitor of the amphetamine-induced dopamine increase.

In the current thesis, a single oral dose of 0.5 mg/kg amphetamine was used to measure amphetamine-induced displacement of $[^{18}\text{F}]$fallypride binding in striatal and extrastriatal regions. After oral administration of this dose in normal subjects, plasma concentrations peaked at 3–4 h, which was approximately twice that seen in a group of subjects administered 0.25 mg/kg (Angrist et al 1987). Maximum subjective and behavioural effects of oral amphetamine have occurred at 1.5–3 h (Angrist et al 1987; Asghar et al 2003; Brauer et al 1996). Amphetamine is eliminated in adults with a mean terminal half-life of about 13–14 h (Martinsson et al 2003). Over recent years, oral amphetamine has been shown to robustly displace benzamide $D_2$ radioligand binding in
healthy subjects (see Chapter 1). Recently, 0.43 mg/kg oral amphetamine displaced [\(^{18}\text{F}\)]fallypride in healthy humans, but only by 3 – 11% (Riccardi et al 2006). As such, a slightly higher dose of 0.5 mg/kg was administered in Study 1 of the current thesis, with an upper limit of 46.5 mg per subject as set by the exclusion of subjects over 93 kg for AMPT administration (see 4.4.2 below). This dose is around the limit of that used in children and adult patients with attention deficit hyperactivity disorder.

Methylphenidate is another stimulant that elevates synaptic dopamine levels but, in contrast to amphetamine, achieves this through blocking DAT-mediated reuptake of dopamine only (Izenwasser et al 1999; Pan et al 1994). Methylphenidate has also been used to measure *in vivo* changes in synaptic dopamine release through competition binding studies (see Chapter 1). However, amphetamine-induced dopamine release shows better reproducibility than methylphenidate-induced release (Kegeles et al 1999; Wang et al 1999) and as mentioned above, oral administration of amphetamine has consistently been found to displace benzamide radioligands, whereas the effect of oral methylphenidate is less robust (refer to Chapter 1).

4.4.2 Pharmacological depletion of dopamine transmission with AMPT

The second pharmacological manipulation used in Study 1 of the current thesis was with AMPT. In contrast to amphetamine, which was administered to elevate synaptic dopamine levels, AMPT was used to deplete dopamine levels for subsequent estimation of baseline or ‘tonic’ dopamine transmission. As outlined in Chapter 1, the first and rate-limiting step in catecholamine biosynthesis is the hydroxylation of tyrosine to L-dopa by the enzyme tyrosine hydroxylase. AMPT selectively inhibits catecholamine synthesis by competitively inhibiting tyrosine hydroxylase (\(K_i = 17\mu\text{M}\)), and thus deplete catecholamines in tissue (Engelman et al 1968; Sjoerdsma et al 1965; Spector et al 1965). AMPT is commercially available (Demser®) and approved for the treatment of pheochromocytoma. Peak concentrations of AMPT in plasma (12–14 µg/mL) occur about 2 hours after ingestion of a single dose of 1 g AMPT and decline with a half-life of about 3.5 – 4 h. These kinetics predict concentrations of 19–22 µg/mL at steady-state with administration of AMPT every 6 h (Engelman et al 1968). Maintaining similar plasma concentrations of AMPT for 24 h reduced catecholamines to nondetectable
levels in the caudate nucleus of guinea pigs (Spector et al 1965) and depleted cerebrospinal fluid catecholamines by about 68−77% (Brodie et al 1971). Administration of 1 g AMPT every 6 hours for 2 days in healthy subjects resulted in an average AMPT plasma concentration of 21 µg/mL (Laruelle et al 1997a). Based on a simulated relationship between AMPT plasma concentration and tyrosine hydroxylase inhibition, using a plasma tyrosine concentration of 10 µg/mL, and an affinity constant for tyrosine hydroxylase of 17 µM for AMPT and 62.5 µM for tyrosine, Laruelle et al (1997a) estimated that this mean plasma concentration would have achieved 78% inhibition of the enzyme. A similar concentration of AMPT is expected in Study 1 of the current thesis with the proposed AMPT regime (3g/70 kg per day for 2 day).

In a seminal study of the field, Laruelle et al (1997a) demonstrated that AMPT treatment significantly increased striatal [123I]IBZM binding in a group of healthy subjects. In rodents, administration of a significantly higher dose of AMPT for two days did not affect D2 receptor B\text{max}, indicating that the increased radioligand binding was not due to D2 receptor upregulation but by the removal of endogenous dopamine and unmasking of D2 receptors (Laruelle et al 1997a). Baseline occupancy of D2 receptors by dopamine was estimated at 21%. A number of studies have since shown AMPT-induced effects on \textit{in vivo} radioligand binding in striatal and some extrastriatal regions (Abi-Dargham et al 2000b; Fujita et al 2000; Riccardi et al 2008; Verhoeff et al 2003; Verhoeff et al 2002; Verhoeff et al 2001), as outlined in Chapter 1.

Depletion of the amino acids tyrosine and phenylalanine is another method used to deplete dopamine levels in the brain (McTavish et al 1999; Milner and Wurtman 1986; Tam and Roth 1997). However, the extent of dopamine depletion achieved with this amino acid depletion is likely too small (increasing striatal [11C]raclopride binding by only 6%) (Montgomery et al 2003) and too variable (Mehta et al 2005) to reliably assess tonic dopamine function in striatal and extrastriatal brain with \textit{in vivo} imaging techniques. As such, the more aggressive and reliable dopamine depletion method of AMPT was used to modulate the \textit{in vivo} binding of [18F]fallypride in the current thesis.
4.4.3 “Pathological” dopamine measurement: Parkinson disease and the dopamine system

Progressive degeneration of brain nuclei dopaminergic neurons as well as the appearance of intracytoplasmic inclusions known as Lewy bodies represents the primary neuropathology in idiopathic PD (Jellinger 2001; Takahashi and Wakabayashi 2001). This loss of dopamine neurons occurs predominately in the nigrostriatal tract but to a lesser extent in the mesocorticolimbic tract (Jellinger 2001), and leads to a chronic and uneven deficiency of dopamine in the striatum (Ehringer and Hornykiewicz 1960; Hornykiewicz and Kish 1987; Kish et al 1988), and other brain areas in the course of the disease (Kaasinen and Rinne 2002; Scatton et al 1983).

Clinically, PD is characterized by the classical symptom triad of rigidity, bradykinesia and resting tremor, which are related to the progressive loss of nigrostriatal dopamine (Fahn 2003) and subsequent abnormalities in neural (i.e. “motor”) circuitry (Wichmann and DeLong 1993). At the point of clinical expression the pathology of PD has reached an advanced stage, where there is about a 50% loss of nigral dopaminergic neurons and an 80% reduction of dopamine concentration in the putamen (Braak et al 2003), which, in vivo, translates into about a 50% reduction of \(^{[18]F}\)FDOPA uptake in the caudal putamen (refer to Chapter 1). The severity of rigidity and bradykinesia has repeatedly been related to both nigral (especially ventrolateral) neuronal loss (Rinne 1991; Rinne 1993; Rinne et al 1989) and striatal dopamine deficiency (Bernheimer et al 1973; Brooks and Piccini 2006; Kaasinen and Rinne 2002; Vingerhoets et al 1997). Striatal depletion is proposed to disrupt the neural circuitry within the basal ganglia that regulates movement (refer to Chapter 1) via inhibition of the “direct” and over-activity of the “indirect” pathways of the motor circuitry (Wichmann and DeLong 1993). In addition to the classic motor symptoms, non-motor symptoms are also prevalent in PD, including cognitive impairment and dementia (Bodis-Wollner 2003; Caballol et al 2007; Owen 2004; Zgaljardic et al 2003), neuropsychiatric features (such as depression, anxiety and apathy) (Marsh 2000; McDonald et al 2003; Richard 2005), as well as sleep, autonomic and sensory dysfunctions (Comella 2006; Micieli et al 2003; Ziemssen and Reichmann 2007), some of which may, in part, be related to the dopaminergic pathology of the disease. Cognitive impairment, particularly in executive-type processes but also dementia, has been associated with deficits in in vivo molecular markers of the dopamine system in PD, as was reviewed in Chapter 2 of this thesis. In addition to these
in vivo associations, dementia in PD has been related to neuronal loss in the medial substantia nigra (Rinne et al 1989) as well as greater cortical dopamine depletion than non-demented patients (Scatton et al 1983).

As mentioned above and in Chapters 1 and 2, the neurochemical pathology in PD is not even, with neuronal degeneration, striatal dopamine depletion and consequently the underlying frontostriatal circuitry following a characteristic spatio-temporal progression, which may well account for the distinct profile of cognitive sequelae and the contrasting effects of L-dopa treatments seen in PD (Cools 2006; Kaasinen and Rinne 2002; Owen 2004). In brief, dopamine cell loss progresses from the ventrolateral tier of the substantia nigra to dorsal-medial components (Rinne 1993). In line with the topographical organisation of the substantia nigra (with ventrolateral aspects projecting to the dorsal striatum and dorsal-medial aspects to the ventral striatum), dopamine depletion is more severe in dorsal than ventral striatum (Bernheimer et al 1973; Kish et al 1988). Within the dorsal striatum, the dorsal caudal portion of the putamen is first affected, with depletion extending to rostrocaudal portions of the caudate (i.e. dorsolateral head) and ventral putamen as the disease progresses (Kish et al 1988; Morrish et al 1996b). In comparison, depletion in the ventral caudate is not apparent until later stages of the disease. Consequently, the frontostriatal neuronal circuits, as well as the behaviours they subserve (see section 1.3.3) are proposed to be differentially affected in PD. That is, the “motor” loop is more severely altered (accounting for the primary motor symptoms) followed by the “DLPFC” circuit (see Figure 1.6 in Chapter 1), which likely contributes to the prefrontal-type cognitive deficits seen in even early PD, and the alleviation of these deficits by L-dopa more so at early stages (Cools 2006). As discussed in Chapter 2, however, depletion within the mesocortical dopamine pathway may also contribute to these “prefrontal” or “frontostriatal” cognitive impairments in PD.

Although PD is primarily considered a dopaminergic disorder, it is important to acknowledge that non-dopaminergic pathology is also involved (see Ahlskog 2007; Hodaie et al 2007). In particular, abnormalities in other neurochemical systems, such as the serotonin (Guttman et al 2007; Kish et al 2008; Scatton et al 1983), noradrenaline (Fornai et al 2007; Scatton et al 1983), cholinergic (Hilker et al 2005; Ruberg et al 1986) and gamma-aminobutyric acid (GABA) (Galvan and Wichmann 2007; Kish et al
1986) systems have been observed in PD. Such neurochemical alterations, as well as other non-dopaminergic features (e.g. cortical Lewy bodies) may play a role in some of the non-motor aspects of PD, including some cognitive deficits and dementia (Emre 2003; Hurtig et al 2000; Williams-Gray et al 2006).

In light of the above background, Study 2 of the current thesis used patients with idiopathic PD to represent (albeit indirectly) chronic, non-pharmacological dopamine depletion, and examined the effect of PD (and as such chronic dopamine deficiency) on pre- and post-synaptic dopamine markers and cognitive function within the frontostriatal human circuitry. The frontostriatal cognitive processes were selected to primarily reflect the neural circuit connecting the dorsal striatum and PFC (predominately DLPFC), which would be expected to be severely depleted in the sample of PD patients of the current thesis.

### 4.5 PARTICIPANTS

#### 4.5.1 General inclusion and exclusion criteria

The two experimental studies in the current thesis sampled markedly different populations of participants; namely young healthy subjects who underwent pharmacological manipulations, and older subjects diagnosed with idiopathic PD and age matched controls. The young sample of Study 1 restricted age between 18 and 45 years. An upper limit of 45 years was chosen to minimize the effect of age on D2 receptors (detailed in Chapter 1) and because of the possibility that amphetamine adversely affect subjects at risk for cardiovascular disease. The minimum and maximum age of PD and control participants in Study 2 was 40 – 85 years, consistent with the older demographics of PD patients. None of the participants in either study were smokers. With the exception of one participant (male healthy control in Study 2), all were right-hand dominant. All participants provided written informed consent before participating in the study.

Details of the sample criteria for each study are described in their respective experimental chapters. In general, all participants were in good health at the time of testing and free of medical, neurological and psychiatric conditions, with the exception
of conditions directly relating to PD. All participants were asymptomatic (no headache, dizziness, neurological symptoms or blurred vision) and showed normal sustained blood pressure at baseline evaluation on the day of each PET scan. General exclusion criteria included pregnancy or lactation, history of an abnormal MRI, high blood pressure (appropriate for younger and older subjects, see each experimental chapter), medically significant biochemical or hematological abnormality on screening laboratory tests, substance abuse and current medical, neurological and psychiatric illness appropriate for each sample population. Further, participants were excluded if their total research based radiation exposure within the previous 12 months in addition to the current study exceeded a total effective dose of 5 rem. Eligibility for the study was ascertained through a telephone screener interview, followed by a clinical evaluation performed by a credentialed physician or credentialed nurse practitioner, which included a physical and neurological examination, routine laboratory tests (including a complete blood count, chemistries, thyroid function test, serum electrolytes, liver and kidney function, urinalysis, urine drug screen and HIV and Hepatitis B tests), electrocardiogram and history. A pregnancy test (via urine or blood sample) was performed on all female subjects of child bearing potential at screening and within 24 h before each PET scan. In Study 2 a semi-structured clinical interview based on the DSM-IV was also conducted.

4.6 PET AND MRI SCANNING PROTOCOL

All studies were conducted at the National Institutes of Health Clinical Center, Bethesda, Maryland. PET scans were acquired in the three-dimensional mode using a GE Advance tomograph (GE Medical Systems, Milwaukee, WI). The anatomic resolution, measured as the full-width at half-maximum, of this camera is about 5 mm in all three directions. For all PET scans, the subject was placed on the scanner bed in a supine position with their head held firmly in place (to minimize head movement) with a thermoplastic mask fixed to the bed. Markers on the mask were used to re-orient the PET image to a standard plane. In all PET studies, an 8-min transmission scan using a $^{68}$Ge rotating pin source was performed prior to tracer injection to provide a measured correction of photon attenuation of the head. Each PET tracer was injected intravenously over 1 min through an antecubital venous catheter. Emission data were acquired in 35 simultaneous slices with a 4.25 mm inter-slice distance. Subjects were
monitored during the PET scan and had access to trained health professionals at all times. In addition, all subjects received a 1.5 Tesla MRI scan to obtain high resolution anatomical images of the brain. These were acquired for image coregistration, segmentation and identification of anatomical regions, as well as for confirming an absence of a neurological abnormality. The MRI protocol acquired three different contrast images. These were: inversion recovery fast gradient recalled-echo (IR-FGRE; TR ~12 msec, TE ~5 ms, flip angle 20°, voxel size: 0.86 × 0.86 × 1.2 mm), fast spin echo (FSE) T2-weighted (TR ~3700 ms, TE ~101 ms, flip angle 90°, voxel size: 0.43 × 0.43 × 5 mm) and fluid attenuated inversion recovery (FLAIR; TR ~10002 ms, TE ~140 ms, flip angle 90°, voxel size: 0.86 × 0.86 × 5 mm) images. Some MRI scans were repeated if the image quality was inadequate due to subject movement or for other reasons.

4.7 GENERAL DATA ANALYSIS

Two data analysis packages, PMOD 2.65 (pixel-wise modeling computer software; PMOD Technologies Ltd, Adliswil, Switzerland; [http://www.pmod.com/technologies/index.html](http://www.pmod.com/technologies/index.html)) and statistical parametric mapping 2 (SPM2, The Wellcome Department of Cognitive Neurology, London, UK; [http://www.fil.ion.ucl.ac.uk/spm/](http://www.fil.ion.ucl.ac.uk/spm/)) were primarily used for the analysis of dynamic PET images. These image analysis packages are available commercially and are widely used for PET analysis. Various aspects of image preprocessing, image display and viewing, quantitative modeling, correction methods and statistical analyses were performed with PMOD and SPM2. Other software packages and analysis tools, such as FMRIB’s linear image registration tool (FLIRT), were used when appropriate. Specific details of the image analysis procedure, including pre-processing steps, are provided within each experimental chapter. For both studies however, MRI (IR as well as T2 and FLAIR images) were coregistered to PET data. Segmentation of each individual’s MRI into gray matter, white matter and cerebrospinal fluid images was performed using multispectral image processing with the three MRI contrast images. All PET imaging data underwent both region of interest (ROI) and voxel-wise analysis after appropriate tracer kinetic modeling for parameter estimation. The region of interest approach was used to test hypotheses about specific regions. The voxel-by-voxel approach, which performs
statistical analyses for every voxel, was used to explore the location of possible effects outside the selected regions. With the exception of striatal subdivisions, template-based methods were used for extraction of the regions of interest data. This approach involves defining regions on a template which all subjects have been normalized to, or which has been normalized to each individual subject (Sun et al 2007). We chose the template-based approach over the subject-specific approach because it is less time and labour intensive and because it is not subject to the variability and potential bias of subjectively drawing regions on each individual’s MRI. Data extracted from template and subject-specific regions have shown to be highly correlated in young, healthy subjects (Yasuno et al 2002) and in older subjects given use of an appropriate template (Sun et al 2007). Because both template-based regions of interest and voxel-based approaches assume that each pixel or voxel corresponds to the same anatomical region across subjects (i.e. all subjects are in the same spatial space), their results are dependent on the accuracy of the stereotaxic normalization process (Sun et al 2007). This accuracy can be affected by the choice of template (i.e. target image) to which all subjects are normalized, particularly for aging and patient populations. In the current thesis, stereotaxic normalization (for both region and voxel-based methods) was improved by use of appropriate templates for each study population. In Study 1, which consisted of young, healthy subjects, PET and MRI images were spatially normalized to the Montreal Neurological Institute (MNI) gray-matter apriori template. The MNI template is the international standard according to the International Consortium of Brain Mapping and was created from MRI scans of normal subjects (reviewed in Brett et al 2002). Study 2, which consisted of older subjects (both healthy and patient), showed substantial errors in spatial normalization using the standard MNI template, likely because of noisy MRI data. For older subjects, a template derived from an older cohort was more accurate at extracting PET data than a standard template derived from normal young subjects (Sun et al 2007). In Study 2, a custom template composed of the same subjects used in the study was created for final spatial normalization, which was therefore more representative of the study cohort than the MNI template.

4.8 PARTIAL VOLUME CORRECTION

As discussed in section 4.1.3 partial volume effects are a frequent problem encountered
in PET imaging common to the analysis of structures that are small or contaminated by surrounding tissue, including cerebrospinal fluid, white matter or bone. Correction of partial volume effects was performed in the current thesis with use of an MRI-based approach (Muller-Gartner et al 1992). Partial volume correction is applied to PET data mainly for two reasons: 1) to restore loss of signal to small regions; in the current thesis this was related to restoring the loss of signal to striatal subdivisions in Study 1, and 2) to minimise or correct for activities arising from non-gray matter tissue on the PET signal. In the current thesis, partial volume correction was used to reduce contamination of gray matter by radioactivity in underlying white matter.

Partial volume correction was performed in PMOD using a model based on Giovacchini et al (2004). This MRI-based approach utilises three segments of MRI – gray matter, white matter and cerebrospinal fluid (CSF), which are all in the same spatial space as the original uncorrected PET image, to estimate the true gray matter tracer concentration subjected to partial volume effects (Muller-Gartner et al 1992). Partial volume correction involves several steps and generates several intermediate parameters (Giovacchini et al 2004) (Figure 4-4). First, the MRI segments create probability images for gray matter, white matter and CSF, where voxel intensity represents the probability of the tissue group. The probability images for gray and white matter are then smoothed with a Gaussian kernel according to the spatial resolution of the PET scanner. These smoothed images represent the fraction in the voxel that is gray and white matter, respectively. The model assumes that activity in the CSF is zero while activity in white matter is uniform. Partial volume correction involves correcting the gray matter voxels for spill-out of activity and for spill-in of activity from white matter. In essence, this is calculated by subtracting the estimated white matter activity from the uncorrected image and dividing it by the smoothed gray matter image (Figure 4-4). To accurately estimate the white matter concentration for subtraction, pixel values that represent “pure” white matter are required. In this model, this value is automatically estimated for each frame; first by identifying the activity values of pixels with a white matter membership greater than 99% (i.e. unaffected by gray matter or CSF activity), and second, by using linear regression, extrapolating to pixels that represent 100% white matter, with the activity value of these pixels used as the white matter concentration (Giovacchini et al 2004). No operator interaction is required for pure white matter identification. In the current thesis, this PVC calculation was applied to every PET frame (of each of \[^{18}\text{F}]\text{fallypride},
[\textsuperscript{11}C]NNC 112 and [\textsuperscript{18}F]FDOPA scans) to generate an activity image corrected or minimized for partial volume effects.

**Figure 4-4.** Schematic diagram of the steps involved in MRI-based partial volume correction of PET activity data. The resultant images in order of processing are: a) original uncorrected PET image; b) coregistered MRI image; c) segmented gray matter image; d) segmented white matter image; e) smoothed white matter image; f) smoothed gray matter image; g) PET gray matter image obtained by subtracting e from a; and h) the corrected PET image obtained by dividing g by f. Diagram reproduced from Meikle and Badawi (2005).

### 4.9 STATISTICAL ANALYSIS

All statistical analyses are detailed within the methods section of each experimental chapter. For both experimental studies, analysis of clinical, cognitive and PET region of interest data used the Statistical Package for the Social Sciences (SPSS) (SPSS Inc., 1989–2004, Release 13.0). Data were analysed with parametric statistics if the assumption of normality, as based on the Shapiro-Wilk test, was met. Non-parametric analysis was used when the assumption of normality was violated. Statistical analysis of voxel-based PET data was performed with SPM2, following standard procedures for between and within-group comparisons of PET data.
4.9.1 Multiple comparison control

As mentioned in Chapter 2, relationships between regional PET indices and specific cognitive processes have often been reported without adequate control for the number of comparisons performed. These studies are, therefore, at risk of making type I errors (i.e., rejecting the null hypothesis when it is actually true). Of studies that did control for multiple tests, the significance threshold was generally submitted to a traditional Bonferroni correction, which although has a conservative type I error rate, has an increased chance of committing a type II error (i.e., failing to reject the null hypothesis when it is false). This multiplicity issue is pertinent to the experiments of the current thesis, as although statistical comparisons were made for several brain regions and cognitive measures, some were made a priori. In the current thesis, a more powerful multiple testing approach was adopted which controls for multiple comparisons with a false discovery rate (FDR) correction (Benjamini and Hochberg 1995). This modified Bonferroni procedure controls the expected proportion of falsely rejected hypotheses without significantly reducing the statistical power (and hence inflating the type II error). In brief, the FDR correction involves ordering the $P$ values ($P_i$s) for the number of comparisons made from highest to lowest. Controlling the false discovery rate at 0.05, each $P_i$ is compared sequentially with $0.05i/m$, with $m$ being the number of comparisons made. This step-down procedure is continued until a $P$ value satisfies the constraint, and subsequently all hypotheses below this $P$ value are also rejected. Control of the FDR has been demonstrated to be considerably more powerful than traditional methods that control the family-wise error rate, such as the Bonferroni correction (Benjamini and Hochberg 1995). Voxel-based imaging data was likewise submitted to FDR correction according to standard procedures in SPM2.
Chapter Five

5 Study 1: PET imaging of dopamine D\(_2\) receptors and extracellular dopamine with \([^{18}\text{F}]\text{fallypride, } d\)-amphetamine, and alpha-methyl-para-tyrosine in healthy subjects: exploration with cognition

5.1 INTRODUCTION

As discussed in Chapter 1, molecular imaging with SPECT or PET can be used not only to measure D\(_2\) receptor density, but also, under appropriate conditions, to estimate synaptic concentration of endogenous dopamine. This approach is based on the competition between certain radioligands and endogenous dopamine for D\(_2\) receptor binding, according to pharmacological theories defined by an occupancy model (Laruelle 2000), as previously detailed in preceding chapters. Over the past decade, SPECT or PET D\(_2\) receptor measurement coupled with pharmacological interventions either to increase synaptic dopamine levels with a stimulant acutely or deplete dopamine levels rapidly, have been used to examine synaptic dopamine transmission in human brain (for review see Chapter 1). However, the majority of these studies have been confined to the striatum, since the striatum is a relatively large region with an abundance of D\(_2\) receptors (Kessler et al 1993). Stimulating dopamine release with either intravenous or oral doses (0.3 mg/kg or 30 mg) of amphetamine in healthy subjects has consistently decreased striatal binding of \([^{11}\text{C}]\text{raclopride (Boileau et al 2006; Cardenas et al 2004; Drevets et al 2001; Leyton et al 2002; Martinez et al 2003)}\) and \([^{123}\text{I}]\text{IBZM (Kegeles et al 1999; Laruelle et al 1995)}\) by about 7 – 18%. In contrast, depletion of cerebral dopamine with AMPT (Engelman et al 1968), a competitive inhibitor of tyrosine hydroxylase, increased \([^{11}\text{C}]\text{raclopride and } [^{123}\text{I}]\text{IBZM binding in striatum by 9 – 28\% (Abi-Dargham et al 2000b; Laruelle et al 1997a; Verhoeff et al 2003; Verhoeff et al 2002; Verhoeff et al 2001)}\). Increase in radioligand binding was suggested to be due to removal of endogenous dopamine, subsequently unmasking D\(_2\) receptors previously occupied by it and thus providing a measure of baseline or tonic dopamine release (Laruelle et al 1997a).
Although assessment of striatal dopamine release is important for increasing our understanding of a number of psychiatric and neurological disorders, a number of studies indicate the involvement of extrastriatal dopamine transmission in schizophrenia, addiction, neuroleptic drug interactions and various cognitive processes (Arnsten 1998; Laviolette and Grace 2006; Lidow et al 1998). Recent focus has been directed to developing high-affinity SPECT and PET radioligands that enable quantification of low-density extrastriatal dopamine receptors. $^{[18F]}$fallypride is a high-affinity D$_2$/D$_3$ radioligand ($K_D = \sim 0.2$ nM, \textit{in vivo} or 30 pM, \textit{in vitro}) (Mukherjee et al 1995; Slifstein et al 2004a) which, with its high specific-to-nondisplaceable binding, is capable of measuring D$_2$-type receptors in striatal, as well as extrastriatal regions such as thalamus, temporal cortex, substantia nigra and limbic areas (Mukherjee et al 2002). $^{[18F]}$Fallypride is sensitive to changes in extracellular levels of endogenous dopamine, both in the striatum and extrastriatal regions. In non-human primates, a 14 – 49% displacement of $^{[18F]}$fallypride was reported in the striatum, thalamus, amygdala, hippocampus and pituitary, following 0.6 – 1 mg/kg intravenous dose of amphetamine (Mukherjee et al 2005; Mukherjee et al 1997; Slifstein et al 2004b). Recently, amphetamine-induced displacement of $^{[18F]}$fallypride binding was demonstrated in both striatal and extrastriatal regions in healthy volunteers following an oral dose of 0.43mg/kg amphetamine (Riccardi et al 2006). Displacement of $^{[18F]}$fallypride was greatest in striatal subdivisions (6–11%) and substantia nigra (7%), with lesser displacement seen in amygdala, temporal cortex and thalamus (3–4%). As detailed in Chapter 2, relationships between regional amphetamine-induced dopamine release and cognitive tasks assessing cognitive processing speed, attention and response inhibition were also observed. In addition, depletion of dopamine with AMPT was recently reported to increase $^{[18F]}$fallypride binding by 9–13% in striatal subdivisions and substantia nigra in a small group of healthy volunteers (Riccardi et al 2008). Although preliminary findings suggest that $^{[18F]}$fallypride is vulnerable to changes in endogenous dopamine levels in the striatum and extrastriatal areas of healthy humans, changes in extrastriatal regions have been relatively small, and not all extrastriatal regions have shown significant effects. Therefore, this study aimed to determine whether amphetamine- and AMPT-induced changes in $^{[18F]}$fallypride binding are reproducible, and evident across a wider range of extrastriatal regions. Further, to date no study has examined the effects of drug-induced and “tonic” dopamine release on striatal and extrastriatal D$_2$ receptors within the same subjects. Although drug-induced dopamine...
release may be different from physiological phasic release, given the close relationship between these two modes of dopamine transmission, with tonic release modulating phasic release (Grace 1991), we wanted to establish the effect of within-subject changes in both amphetamine-induced and tonic dopamine on $[^{18}\text{F}]$fallypride binding. Such within-subject examination may aid in understanding the interactions between phasic and tonic dopamine release.

This study performed $[^{18}\text{F}]$fallypride PET scans at baseline and following oral amphetamine and AMPT administration in healthy human volunteers. The purpose of the study was to; 1) assess the reproducibility of measuring $[^{18}\text{F}]$fallypride binding (i.e. test-retest reliability), 2) for the first time, examine within the same subjects, the effects of amphetamine-induced dopamine release and AMPT-induced “tonic” dopamine depletion on $[^{18}\text{F}]$fallypride binding in both striatum and extrastriatal areas, and 3) examine the within-subject relationship between amphetamine-induced and tonic dopamine release as measured by $[^{18}\text{F}]$fallypride binding in both striatum and extrastriatal areas, and their relationship with cognition. Based on previous studies and the hypothesis put forth by Grace (1991), it was hypothesized that amphetamine and AMPT pretreatment would decrease and increase $[^{18}\text{F}]$fallypride binding, respectively, and that subjects with greater amphetamine-induced release would have smaller tonic release. This study has been published in Synapse (Cropley et al 2008) (see Appendix 2 for reprint).

5.2 METHODS

5.2.1 Study population

Fourteen healthy volunteers (11 male, 3 female; mean age ± SD, 29 ± 8 y; range, 20 – 43 y) participated in the study. Subjects were free of current medical, psychiatric and neurological illness based on history, a physical exam, laboratory tests and electrocardiogram. Further exclusion criteria included elevated blood pressure (>140/90), history of myocardial infarction or angina pectoris, positive urine drug screen, history of substance abuse or dependence within 6 months and body weight greater than 93 kg. The Radiation Safety Committee of the National Institutes of Health and the Institutional Review Board of the National Institute of Mental Health approved
the study. All subjects gave written informed consent.

5.2.2 Radiopharmaceutical preparation

\[^{18}F\]Fallypride was synthesized via nucleophilic substitution of tosyl-fallypride (ABX GmbH, Radeberg, Germany) with cyclotron-produced \[^{18}F\]fluoride ion (Mukherjee et al 1995). Preparations were conducted according to the Molecular Imaging Branch (NIMH, Bethesda, Maryland) Investigational New Drug Application #70,046, submitted to the US Food and Drug Administration and a copy of which is available at: http://kidb.bioc.cwru.edu/snidd/. In brief, synthesis was carried out in a modified TracerLab FX F-N (GE Healthcare). The radiochemical purity for all batches was greater than 95%, as assessed by radio- high performance liquid chromatography (HPLC). Specific radioactivity was determined by radio-HPLC calibrated for absorbance response at 305 nm to mass of fallypride. Chemical contaminants represented less than 0.4 mg equivalent of fallypride in all lots. Formulated \[^{18}F\]fallypride was radiochemically stable for the duration of all experiments.

5.2.3 Scanning protocol

PET scans were performed using a GE Advance tomograph and were reconstructed with the filtered-back projection algorithm which resulted in a final image resolution of 7.5 mm FWHM. Following the initial screening visit, subjects underwent four \[^{18}F\]fallypride PET scans on four separate days in the following order – two baseline scans (test-retest) (scan 1 and 2), a scan with amphetamine administration (scan 3) and a scan with AMPT administration (scan 4). The median interval between baseline test-retest scans was 2 weeks, while there was about a 1 month interval between scan 2 (retest) and scan 3 (amphetamine) and 6 weeks between scan 3 (amphetamine) and scan 4 (AMPT) (Table 5-1). In all PET studies, a transmission scan was performed for attenuation correction. Dynamic emission scans were acquired following an intravenous bolus injection of 187 ± 11 MBq \[^{18}F\]fallypride for 3 h (specific activity = 70 ± 37 GBq/\mu mol; mass dose/body weight = 0.017 ± 0.008 \mu g/kg). The initial image acquisition coincided with \[^{18}F\]fallypride injection and was obtained continuously for 60 min (6 × 30 s, 3 × 1 min, 2 × 2 min, 10 × 5 min). Following this initial acquisition,
two 1-h images (12 × 5 min) were acquired until about 5 h after the bolus injection. Subjects were removed from the camera for about 60 min between image acquisitions. To ensure metabolism of the radioligand was consistent among scans, subjects did not eat from 2 h before [18F]fallypride administration to the completion of the scan, and half normal saline was intravenously infused at a rate of 83 mL/h starting 1 h before [18F]fallypride injection. Subjects also consumed at least 1 L of water before and during intermissions of the PET scan. To minimize potential changes in dopamine levels, subjects were asked to refrain from consuming caffeine-containing drinks after midnight before each PET scan.

On the day of PET scan 3, a single oral dose of 0.5 mg/kg amphetamine (Dexedrine®) was administered 3 h before injection of [18F]fallypride with vital sign monitoring. Scanning was performed 3 h following amphetamine administration because there is sustained radioligand displacement following oral amphetamine (Cardenas et al 2004). A slightly higher dose of amphetamine (0.5 mg/kg) was used than in previous studies (0.3 – 0.43 mg/kg) to induce greater dopamine release and to more easily detect changes in [18F]fallypride binding. Plasma amphetamine levels were measured at about 3 and 4.5 h following amphetamine administration to measure amphetamine levels during the PET scanning period. Plasma samples were analyzed for amphetamine using an isotope dilution procedure. The method was a minor modification of the procedure of Xie et al (2004). The modification for plasma involved initial protein precipitation with sulfosalicylic acid. The clear supernatant was then processed as described for ultrafiltrates in Xie et al (2004). The standard curve was linear (r = 0.999) in the range tested (1–500 ng/mL). The limit of detection was 1ng/mL with intra and inter coefficients of variation of <5% and <7% respectively.

For PET scan 4, subjects were administered 3g/70 kg body weight AMPT (Demser®) orally per day over 44 hours (10 doses in total) (Fujita et al 2000; Laruelle et al 1997a). Although Laruelle et al (1997a) did not adjust AMPT dose based on body weight, they administered almost the same dose for the same length of time. Subjects with body weight more than 93 kg were excluded from the study to limit AMPT dose to 4 g/day. The AMPT component of the study was performed over four days with three overnight stays at the NIH Clinical Centre. Subjects were admitted to the Centre on day 1 and received AMPT doses at 6 PM and 11 PM. On day 2, subjects received AMPT at 7 AM,
12 PM, 6 PM and 11 PM while on day 3, subjects received AMPT doses at 7 AM, 9 AM, 12 PM and 2 PM. The total dose of AMPT remained at 3 g/70 kg/day. The PET scan was performed on day 3, after the 9 AM dose. Subjects remained overnight at the Clinical Centre following the PET scan and were discharged the next day following clinical evaluation and absence of any apparent side effects. To prevent crystalluria during AMPT administration, subjects were asked to drink 1 – 2 L of water each day. In addition, half-normal saline was infused intravenously until 3 h after the end of the fourth PET scan. A urinalysis and medical examination was conducted daily to detect crystal formation and assess tolerance to AMPT treatment. In addition, sodium bicarbonate (2 tablets of 650 mg) was given on three consecutive nights to prevent acidification of the urine during the night. To determine levels of AMPT in plasma, blood samples were collected about 90 min before, and 90 min and 3.5 h after \([^{18}F]fallypride\) injection. Plasma levels of AMPT were determined by HPLC using a modification of a previously reported method for the determination of plasma tryptophan and kynurenine (Hoekstra et al 2007). In brief, 100 µL plasma samples were deproteinized with 100 µL of 0.7 M perchloric acid after addition of 50 µL of 25% ascorbic acid. Supernates obtained after centrifugation were directly injected on a 15 × 0.46 cm Microsorb C18 column eluted with 96% pH 3.7, 1.5% aqueous acetic acid and 4% methanol delivered at a flow rate of 1 mL/min, and the compounds detected fluorometrically (270/320 nm excitation/emission wavelengths). All specimens were analyzed in a single assay and the compounds determined with within-assay coefficients of variation of less than 5%.

Three subjects participated in a pilot study of only one baseline and one amphetamine scan. Of the eleven subjects who participated in the 4 scan protocol (test, retest, amphetamine and AMPT) six subjects completed the fourth scan with AMPT while two subjects commenced the fourth scan but withdrew before completion, allowing measurement in only extrastriatal areas. Therefore, the total number of subjects was 11 for test-retest, 14 for amphetamine-induced change and 6 and 8 for AMPT-induced change in striatum and extrastriatal areas respectively.

All subjects received a 1.5 Tesla MRI scan for coregistration and segmentation purposes, as outlined in chapter four.
### Table 5-1. Participant demographics and clinical measures

<table>
<thead>
<tr>
<th>Measure</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>29±8</td>
</tr>
<tr>
<td>Education (yrs)</td>
<td>16±2</td>
</tr>
<tr>
<td>Amphetamine plasma level at 3 h after administration (ng/mL)</td>
<td>62±19.2</td>
</tr>
<tr>
<td>Amphetamine plasma level at 4.5 h after administration (ng/mL)</td>
<td>71.5±14</td>
</tr>
<tr>
<td>AMPT plasma level (µg/mL)</td>
<td>20±4.3</td>
</tr>
<tr>
<td>$[^{18}F]$fallypride injected activity (MBq)</td>
<td>187±11</td>
</tr>
<tr>
<td>$[^{18}F]$fallypride specific activity (GBq/µmol)</td>
<td>70±37</td>
</tr>
<tr>
<td>Interval between scan 1 and 2 (test-retest) (days)$^1$</td>
<td>15</td>
</tr>
<tr>
<td>Interval between scan 2 (retest) and 3 (amphetamine) (days)$^1$</td>
<td>31</td>
</tr>
<tr>
<td>Interval between scan 3 (amphetamine) and 4 (AMPT) (days)$^1$</td>
<td>42</td>
</tr>
</tbody>
</table>

$^1$Values are mean ± standard deviation  
$^1$Value is median

### 5.2.4 Neuropsychological tests

On the morning of each scan, subjects were administered a neuropsychological battery to examine executive, attentional, processing speed and frontostriatal processes. On amphetamine days, the commencement of the battery corresponded to 60 – 90 min post amphetamine administration as peak subjective and behavioral effects of oral amphetamine have shown to occur within 1 – 3 h after administration (Angrist et al 1987; Asghar et al 2003). For baseline 1 and amphetamine scans the battery consisted of the Symbol Digit Modality task (speed of processing), spatial span (attention and spatial working memory), Stroop color word test (response inhibition, executive function), Controlled Oral Word Association Test – CFL (verbal fluency, executive function), and the Colors Trail Test (visual attention, sequencing, processing speed,). For baseline 2 and AMPT scans, subjects were administered the Digit Symbol Coding test (speed of processing), Controlled Oral Word Association Test – FAS, Colors Trail Test, Letter-Number Sequencing (working memory) and the Stockings of Cambridge task (planning, frontostriatal function). A brief description of each of these tests is provided below. To minimize practice effects, and because of the unavailability of four alternate versions of the neuropsychological tests, different tests were selected for the scans 1 and 3 versus scans 2 and 4. Tests were administered in a fixed order with alternate forms available for some tests. Similar processes were assessed for each of the drug challenge days. The testing interval was ~8 weeks between the baseline 1 and amphetamine sessions and
~11 weeks between baseline 2 and AMPT sessions. One male and one female subject did not undergo neuropsychological testing following amphetamine treatment due to a previous amphetamine scan cancellation that occurred in close proximity to their retest scan. Two subjects did not complete the Letter-Number Sequencing test and two subjects were not administered the Stockings of Cambridge task due to time constraints on the testing day. Drug-induced changes in subjective mood were assessed using 10-point visual analog scales for euphoria, restlessness, anxiety, drowsiness and alertness and the Profile of Mood States (POMS) (McNair et al 1971). The POMS contains 65 adjectives each of which were rated on a 5-point scale ranging from 0 (not at all) to 4 (very much) and representing six mood dimensions: tension-anxiety, depression-dejection, anger-hostility, fatigue-inertia, vigour-activity and confusion-bewilderment. For amphetamine-induced emotional change, subjects rated these questionnaires pre- and about 70 min post amphetamine administration. For AMPT-induced change, subjects were administered the questionnaires at baseline (on the morning of the retest scan) and following AMPT (at the equivalent point in time on the day of the AMPT scan).

**Symbol Digit Modality Test**

The Symbol Digit Modality Test (Smith 1973) is a measure of information processing speed and attention, which requires the subject to match digits to symbols. Using a reference key, the subject had 90 seconds to pair specific numbers with given geometric figures. The score was the number of correct substitutions in the 90-second interval.

**Spatial span**

The spatial span subtest from the Wechsler Memory Scale III (Wechsler 1997b) was used to measure attention and spatial working memory. In this test, the examiner touches a sequence of blocks in view of the subject, who is then asked to repeat the sequence in the exact order. The task has items that are administered forward for the first half and backward for the second.

**Stroop color word test**

The standardized version of the Stroop color word test (Golden and Freshwater 2002) was used to study response inhibition, attention and executive/frontal lobe function. The
test consists of a word page, a color page and a color-word page, each consisting of 100 items. The word page required the subject to read aloud words that are names of colors as fast as they could in 45 seconds. Likewise, the color page required the subject to name aloud colors (printed as XXXX) as fast as they could in 45 seconds. The color-word page tests the ability of the subject to separate the word and color naming stimuli. This page involved showing words that are the names of colors, although the actual words are printed in a color of ink different from the color name they represent. The subject was asked to name the color of the ink the words are printed in, ignoring the word that is printed. Subjects were to complete as many items as they could in 45 seconds. The color-word score was used as the primary outcome measure.

**Controlled Oral Word Association Test (COWAT)**

Phonemic Controlled Oral Word Association Tests (COWAT) was used for assessing verbal fluency and executive function. These tests assess the ease with which a person can think of words that begin with a specific letter. The mean number of words (excluding proper nouns and multiple words with the same stem but ending with a different suffix) generated in one minute for each of the letters C, F and L (baseline 1 and amphetamine scans) and F, A and S (baseline 2 and AMPT scans) were used.

**Color Trails Test**

The Colors Trail Test (D'Elia et al 1996) is a modification of the Trail Making Test. The task requires the subject to connect a series of numbered colored circles in a specified order as quickly as possible. It contains two parts: color trails 1 and color trails 2. For color trails 1, the subject was required to rapidly connect circles numbered 1 through 25 in sequence. For the color trails 2, the subject was required to rapidly connect numbered circles in sequence, but alternate between the colored backgrounds. The score derived for each trail was the number of seconds required to complete the task. The color trails 2 score and the interference index (colors 2 – colors 1 / colors 1) were used as the primary outcome measures. These measures assess sustained and divided attention, sequencing and alternating skills, as well as simple perceptual tracking and graphomotor skills.

**Digit Symbol Coding**

Digit symbol coding is a subtest from the Wechsler Adult Intelligence Scale III
(Wechsler 1997a) and provides a measure of processing speed. This test is similar to the SDMT, but in this test, the subject copies symbols that are paired with numbers according to a reference key. The score was the number of symbols correctly drawn within the 120-second time limit.

**Letter-Number Sequencing**

The letter-number sequencing subtest from the Wechsler Adult Intelligence Scale III (Wechsler 1997a) was used to measure working memory and attention. This test involves ordering numbers and letters presented in an unordered sequence. The subject is read a combination of numbers and letters and is asked to recall the numbers first in ascending order and then the letters in alphabetical order.

**Stockings of Cambridge**

The Stockings of Cambridge task from the Cambridge Automated Neuropsychological Test Battery (Cambridge Cognition, UK) was used to assess spatial planning and frontostriatal/executive function. This computerized task consists of two arrangements of three colored balls, one in the top half of the screen and the other in the bottom half. Subjects were required to rearrange the balls in the bottom display to match the ‘goal’ arrangement in the top display in the minimal number of moves. A series of problems with increasing difficulty (requiring a minimum of 2 – 5 moves to complete) was given. The number of perfect solutions (across all levels of difficulty) was used as the primary outcome measure.

### 5.2.5 Data analysis

To correct for head movement during the scan, all [$^{18}$F]fallypride frames of each PET scan were realigned to a standard frame using the FLIRT algorithm (Jenkinson and Smith 2001) for the initial image acquisition and SPM2 for the second and third acquisitions. FLIRT was used for the initial image acquisition instead of SPM2 because for the initial set, SPM2 cut the bottom portion of images including the cerebellum. Inversion recovery MRI reoriented to the anterior commissure – posterior commissure (AC-PC) line and PET scans 2, 3 and 4 were each coregistered to an average image of initial [$^{18}$F]fallypride frames from PET scan 1 using SPM2. Coregistered serial PET
scans and MRI were spatially normalized to the Montreal Neurological Institute stereotaxic space using segmented gray matter images created from IR, FLAIR and T2 MRI images in SPM2. Regions of interest defined on the caudate nucleus, putamen, thalamus, medial orbitofrontal cortex, anterior cingulate, temporal cortex, medial temporal cortex, substantia nigra and colliculi were applied to spatially normalized PET images. Medial orbitofrontal cortex, substantia nigra and colliculi volumes were delineated on parametric images and the location of the substantia nigra and colliculi were individually adjusted without knowing the scan identity. Striatal subdivisions were drawn on reoriented MRI and were defined according to the criteria of Mawlawi et al (2001) for the ventral striatum, precommissural dorsal caudate and precommissural dorsal putamen and according to Martinez et al (2003) for the postcommissural caudate and putamen. Partial volume correction was applied to striatal subdivisions in order to recover lost spatial resolution to these regions. Partial volume correction was performed in PMOD 2.65, using a model based on Muller-Gartner et al (1992) and Giovacchini et al (2004), as described in the preceding chapter. Both uncorrected and corrected data from striatal subdivisions underwent region of interest analysis.

Regional $[^{18}F]$fallypride binding potential ($BP_{ND}$) was calculated from 5-h data using the simplified reference tissue model (Lammertsma and Hume 1996) implemented in PMOD by using cerebellum excluding vermis as the reference region. Test-retest variability and percent change in amphetamine and AMPT-induced $BP_{ND}$ were calculated for each region of interest. Test-retest variability was calculated as the absolute difference of baseline scan 1 (test) minus baseline scan 2 (retest) divided by the mean of test-retest and expressed as a percent. Amphetamine or AMPT-induced change in $[^{18}F]$fallypride $BP_{ND}$ was determined as the percent difference in $BP_{ND}$ between the mean baseline (scan 1 and 2) and post-amphetamine (scan 3) or post-AMPT (scan 4) conditions: $\Delta BP_{ND} = \left( \frac{[BP_{ND} \text{ (drug)} - BP_{ND} \text{ (mean baseline)}]}{BP_{ND} \text{ (mean baseline)}} \right) \times 100$.

Changes in cognition from baseline to amphetamine or AMPT conditions were calculated by subtracting baseline values from drug (amphetamine or AMPT) values and dividing by baseline. Drug-induced change in mood was assessed as the difference between pre- (or baseline) and post-drug ratings on each VAS and POMS dimension.

In addition, mean parametric images of changes in $[^{18}F]$fallypride $BP_{ND}$ were calculated (Gunn et al 1997) and voxel-wise analysis of change images restricted to the entire
lateral temporal cortex (i.e. small volume correction) was performed using SPM2 and SnPM, the non-parametric version of SPM. The lateral temporal cortex mask was created by combining superior, middle, and inferior temporal cortices of the anatomical labeling template (Tzourio-Mazoyer et al 2002). This was done because $[^{18}\text{F}]$fallypride showed binding in the entire temporal cortex, and changes in an area in the temporal cortex may have been overlooked by applying regions of interest.

### 5.2.6 Statistical analysis

For 11 subjects who had two baseline scans (test and retest), kinetic analysis was performed for each scan and the average values were used as baseline measurements. To determine the effects of amphetamine or AMPT on $[^{18}\text{F}]$fallypride $BP_{ND}$, repeated-measures analysis of variances (ANOVAs) with condition (baseline, amphetamine or baseline, AMPT) and region as within-subject factors, were performed using SPSS for Windows. Greenhouse-Geisser corrections were used for violations of the assumption of sphericity. Separate ANOVAs were performed for striatal subdivisions and other regions (striatum and extrastriatal regions). When appropriate, paired-sample $t$-tests or Wilcoxon signed ranks tests for parametric and non-parametric data respectively, were performed to determine which regions accounted for the significant effects found on the ANOVA. Parametric and non-parametric variables were determined by the Shapiro-Wilk normality test. Right and left sided differences in regional drug-induced displacement of $BP_{ND}$ were examined with paired $t$-tests or Wilcoxon signed rank tests. Relationships between change in $BP_{ND}$ and change in cognition and mood, and between amphetamine and AMPT-induced change in $BP_{ND}$, were performed with Spearman’s rank correlation. Multiple comparisons were controlled for with a false discovery rate correction (Benjamini and Hochberg 1995).

### 5.3 RESULTS

#### 5.3.1 $[^{18}\text{F}]$Fallypride uptake and $BP_{ND}$

$[^{18}\text{F}]$Fallypride was visualized and quantified in both the striatum and extrastriatal regions such as thalamus, temporal cortex, anterior cingulate and orbitofrontal cortex (Figure 5-1). Binding potential in striatal areas were about 10–30 fold higher than
values in extrastriatal areas.

![Image](image.png)

**Figure 5-1.** Parametric image of $[^{18}\text{F}]$allypride $BP_{ND}$ in a healthy subject

### 5.3.2 Test-retest variability

Test-retest variability of $[^{18}\text{F}]$allypride $BP_{ND}$ was low for most regions, ranging from $\sim 3.8\%$ in the striatum, $\sim 5\%$ in medial and temporal cortex, to 6-8% in thalamus, medial orbitofrontal cortex and substantia nigra. Intraclass correlation coefficient (ICC) was above 0.90 (range: 0.91–0.98) for these regions (Table 5-2). Two regions showed test-retest variability above 10%; these were the anterior cingulate (test-retest = 21.8%, ICC = 0.54) and colliculi (test-retest = 10.9%, ICC = 0.72) (Table 5-2). Given the poor reproducibility and low reliability of $BP_{ND}$ in these regions, the anterior cingulate and colliculi were not included in further analysis of amphetamine and AMPT effects. Test-retest variability was excellent in all striatal subdivisions, ranging from 3 - 5.6% and showing ICC above 96% (Table 5-3). Partial volume correction of striatal subdivisions resulted in poorer test-retest variability of $BP_{ND}$ ($\sim 6\%$) compared to uncorrected striatal subdivisions ($\sim 4\%$).
### Table 5-2. Test-retest reproducibility of measuring $[^{18}\text{F}]$fallypride binding potential ($BP_{ND}$)

<table>
<thead>
<tr>
<th>Region</th>
<th>Test</th>
<th>Retest</th>
<th>Test-retest variability (%)</th>
<th>ICC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caudate</td>
<td>17.1±1.0</td>
<td>17.4±1.1</td>
<td>3.8±0.7</td>
<td>0.98</td>
</tr>
<tr>
<td>Putamen</td>
<td>19.8±1.3</td>
<td>19.8±1.4</td>
<td>3.7±0.8</td>
<td>0.98</td>
</tr>
<tr>
<td>Thalamus</td>
<td>1.61±0.08</td>
<td>1.68±0.08</td>
<td>6.1±1.3</td>
<td>0.91</td>
</tr>
<tr>
<td>Medial orbitofrontal cortex</td>
<td>0.60±0.05</td>
<td>0.64±0.06</td>
<td>6.7±1.8</td>
<td>0.95</td>
</tr>
<tr>
<td>Anterior cingulate</td>
<td>0.55±0.05</td>
<td>0.59±0.05</td>
<td>21.8±3.8</td>
<td>0.54</td>
</tr>
<tr>
<td>Temporal cortex</td>
<td>0.72±0.07</td>
<td>0.72±0.07</td>
<td>4.8±1.2</td>
<td>0.98</td>
</tr>
<tr>
<td>Medial temporal cortex</td>
<td>0.86±0.06</td>
<td>0.90±0.07</td>
<td>5.1±0.9</td>
<td>0.96</td>
</tr>
<tr>
<td>Substantia nigra</td>
<td>1.17±0.09</td>
<td>1.16±0.10</td>
<td>7.7±2.0</td>
<td>0.95</td>
</tr>
<tr>
<td>Colliculi</td>
<td>1.52±0.06</td>
<td>1.44±0.08</td>
<td>10.9±2.6</td>
<td>0.72</td>
</tr>
</tbody>
</table>

ICC: intraclass correlation coefficient  
Data are mean ± SEM

### Table 5-3. Test-retest reproducibility of measuring $[^{18}\text{F}]$fallypride binding potential ($BP_{ND}$) in striatal subdivisions

<table>
<thead>
<tr>
<th>Region</th>
<th>Test</th>
<th>Retest</th>
<th>Test-retest variability (%)</th>
<th>ICC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventral striatum</td>
<td>15.8±1.0</td>
<td>16.0±1.1</td>
<td>3.7±0.6</td>
<td>0.98</td>
</tr>
<tr>
<td>Pre-commissural dorsal caudate</td>
<td>18.3±2.1</td>
<td>17.6±1.1</td>
<td>4.4±1.2</td>
<td>0.96</td>
</tr>
<tr>
<td>Pre-commissural dorsal putamen</td>
<td>19.8±1.2</td>
<td>18.8±1.2</td>
<td>5.6±1.0</td>
<td>0.96</td>
</tr>
<tr>
<td>Post-commissural caudate</td>
<td>13.1±0.9</td>
<td>13.1±0.9</td>
<td>3.0±0.9</td>
<td>0.99</td>
</tr>
<tr>
<td>Post-commissural putamen</td>
<td>19.7±1.2</td>
<td>19.2±1.2</td>
<td>3.3±0.7</td>
<td>0.99</td>
</tr>
</tbody>
</table>

ICC: intraclass correlation coefficient  
Data are mean ± SEM

#### 5.3.3 Amphetamine-induced changes

A repeated-measures ANOVA using condition, region and condition by region as factors showed significant main effects for condition ($F(1,13) = 11, p = 0.006$), region ($F(6,78) = 335 , p < 0.001$) and a significant condition by region interaction ($F(6,78) = 12.4, p = 0.003$). The effect of amphetamine on each region was further examined by paired two-tailed $t$-tests. These tests, with correction for multiple comparisons using the false discovery rate (Benjamini and Hochberg 1995), revealed significant amphetamine-induced decreases in $[^{18}\text{F}]$fallypride $BP_{ND}$ in, by rank order, the substantia nigra (13.1%), medial orbitofrontal cortex (13%), putamen (12.3%), medial temporal cortex...
(7.8%) and caudate (7.6%) (Table 5-4). Lower levels of displacement were seen in the thalamus and temporal cortex (7%) but these did not reach significance. For striatal subdivisions, a repeated-measures ANOVA similarly revealed significant main effects for condition ($F(1,13) = 14.2, p = 0.002$), region ($F(4,52) = 76.9, p < 0.001$) and a significant condition by subdivision interaction ($F(4,52) = 21.2, p < 0.001$). Paired-sample $t$-tests showed significant amphetamine-induced displacement in all subdivisions (Table 5-5), ranging between 8 and 14%. Partial volume corrected data showed almost identical statistical results. Right-left differences in amphetamine-induced displacement was found in the putamen only ($p = 0.007$) with the right side showing greater displacement (19.5%) compared to the left side (12.1%). Analysis of striatal subdivisions revealed that the left pre-commissural dorsal putamen showed greater displacement (15.4%) than the right side (11.8%), while the right post-commissural caudate had greater displacement (10.3%) than the left side (5.6%).

\begin{table}[h]
\centering
\caption{Amphetamine-induced changes in $[^{18}\text{F}] $fallypride binding}  
\begin{tabular}{lcc}
\hline
\textbf{Region} & \textbf{Baseline} & \textbf{Post-Amphet} & \textbf{BP} \\
& $BP_{ND}$ & $BP_{ND}$ & \% Change \\
\hline
Caudate & 17.7±0.9 & 16.3±0.9 & -7.6±2.7* \\
Putamen & 20.4±1.1 & 17.9±1.1 & -12.3±2.8* \\
Thalamus & 1.71±0.07 & 1.60±0.10 & -7.0±3.0 \\
Medial orbitofrontal cortex & 0.65±0.05 & 0.56±0.05 & -13.0±4.8* \\
Temporal cortex & 0.79±0.07 & 0.73±0.07 & -7.0±3.1 \\
Medial temporal cortex & 0.94±0.06 & 0.87±0.06 & -7.8±2.8* \\
Substantia nigra & 1.23±0.08 & 1.07±0.08 & -13.1±3.1* \\
\hline
\end{tabular}
\textit{Data are mean ± SEM}  
*Significant change with correction for multiple comparisons using the false discovery rate
\end{table}

Correlations between amphetamine-induced dopamine release and changes in cognition and mood revealed significant negative correlations between changes in the Controlled Oral Word Association Test (CFL) and changes in $BP_{ND}$ in the thalamus (rho = -0.9, $p < 0.001$, corrected for multiple comparisons) and the substantia nigra (rho = -0.9, $p < 0.006$, corrected for multiple comparisons), with greater amphetamine-induced dopamine release being associated with better cognitive performance. No significant correlations were found for other cognitive and mood measures and in other regions.
Voxel-wise analysis with SPM and SnPM did not detect correlations between amphetamine-induced dopamine release and cognitive changes in sub-regions of the lateral temporal cortex.

Plasma amphetamine levels were 62 ± 19.2 ng/mL at ~3 h post administration and 71.5 ± 14 ng/mL at ~4.5 h (Table 5-1).

<table>
<thead>
<tr>
<th>Region</th>
<th>Baseline $BP_{ND}$</th>
<th>Post-Amphet $BP_{ND}$</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventral striatum</td>
<td>16.1±0.8</td>
<td>14.7±0.8</td>
<td>-8.5±2.8*</td>
</tr>
<tr>
<td>Pre-commissural dorsal caudate</td>
<td>18.5±0.9</td>
<td>16.4±0.9</td>
<td>-11.5±2.7*</td>
</tr>
<tr>
<td>Pre-commissural dorsal putamen</td>
<td>20.0±1.0</td>
<td>17.2±1.0</td>
<td>-13.5±2.6*</td>
</tr>
<tr>
<td>Post-commissural caudate</td>
<td>13.6±0.8</td>
<td>12.6±0.9</td>
<td>-7.9±3.0*</td>
</tr>
<tr>
<td>Post-commissural putamen</td>
<td>20.1±1.0</td>
<td>17.4±1.1</td>
<td>-13.6±3.0*</td>
</tr>
</tbody>
</table>

Data are mean ± SEM
*Significant change with correction for multiple comparisons using the false discovery rate

5.3.4 AMPT-induced changes

Repeated-measures ANOVAs in striatal subdivisions and other regions revealed no overall effect of condition or interaction of condition by region. Paired-sample $t$-tests or Wilcoxin signed ranks tests (two-tailed) confirmed that there were no significant changes in $BP_{ND}$ following AMPT treatment in striatal and extrastriatal regions. There was large inter-subject variability in AMPT-induced changes in $BP_{ND}$, with some subjects showing large decreases in binding. Percent change of $BP_{ND}$ ranged between -3.8−1.1% in striatal and -11.7−1.2% in extrastriatal regions. No right/left-sided differences in AMPT-induced change in $BP_{ND}$ were observed. Plasma levels of AMPT remained stable over the course of the PET scan. The average plasma AMPT concentration was 20 ± 4.3 µg/mL (range 14 to 27 µg/mL). Plasma levels of AMPT did not correlate with AMPT-induced change in $BP_{ND}$ in any region.
5.3.5 Correlation between amphetamine and AMPT-induced changes

There were no significant correlations between amphetamine and AMPT-induced changes in $BP_{ND}$ in striatal or extrastriatal regions.

5.4 DISCUSSION

The current study examined the test-retest variability of $[^{18}\text{F}]$fallypride and the effects of changes in amphetamine-induced and tonic (with AMPT) dopamine and their relationship on $[^{18}\text{F}]$fallypride binding in both striatal and extrastriatal regions. This study showed that the reproducibility of $[^{18}\text{F}]$fallypride measurement in striatal and extrastriatal regions was excellent. With the exception of the anterior cingulate and colliculi, the average absolute difference in $[^{18}\text{F}]$fallypride $BP_{ND}$ between test and retest was under 10% for all regions.

Consistent with Riccardi et al (2006), we found that oral administration of amphetamine 3 hours before $[^{18}\text{F}]$fallypride injection significantly reduced radioligand binding to D$_2$ receptors in both the striatum and extrastriatal regions, with the exception of the thalamus and temporal cortex. Amphetamine-induced release ranged between 7 and 14%, with the greatest displacement occurring in the pre- and post commissural putamen, substantia nigra and medial orbitofrontal cortex, and smaller displacement in the caudate, ventral striatum, temporal cortex and thalamus. As expected, the magnitude of this displacement was slightly higher than that reported by Riccardi et al (2006) using $[^{18}\text{F}]$fallypride and a slightly lower dose (0.43mg/kg) of oral amphetamine. Dopamine release in the thalamus and temporal cortex (both 7%) was reasonably greater than the 3–4% displacement observed by Riccardi and colleagues. Nevertheless, these displacements did not remain significant after controlling for the false discovery rate, presumably because of inter-subject variability. Further, the magnitude of regional percent change in the current study exceeds the test-retest variability of $[^{18}\text{F}]$fallypride measurement, indicating that such displacement was not due to poor $[^{18}\text{F}]$fallypride reproducibility.

Although the degree of amphetamine-induced dopamine release was slightly higher than previously reported with $[^{18}\text{F}]$fallypride, the rank order of regional displacement was
similar. As in the study by Riccardi and colleagues (2006), the substantia nigra showed a high level of displacement (13%), which was comparable to that seen in the putamen. Although this is speculative, as discussed by Riccardi and colleagues, such unexpectedly high $[^{18}\text{F}]$fallypride displacement in the substantia nigra may be related to the different proportion of D$_2$ receptors in the substantia nigra and striatum configured in the high- and low-affinity agonist states with a greater proportion of high-affinity state in the former. Another possible explanation is regional differences in the proportion of D$_2$ and D$_3$ receptors (Murray et al 1994) although there has not been a report that fallypride binds preferentially to D$_3$ receptors, which exists with high density in substantia nigra.

With the exception of the ventral striatum, the magnitude of $[^{18}\text{F}]$fallypride displacement in striatal subdivisions was similar to the 8–16% displacement of $[^{11}\text{C}]$raclopride following an intravenous dose of 0.3 mg/kg amphetamine (Drevets et al 2001; Martinez et al 2003). This similarity demonstrates that a relatively high dose of oral amphetamine (0.5mg/kg) is just as effective as an intravenous dose in displacing radioligand binding, and further, that a high-affinity D$_2$ radioligand such as $[^{18}\text{F}]$fallypride, has a comparable sensitivity to competition from changes in amphetamine-induced dopamine release as does $[^{11}\text{C}]$raclopride. There are several benefits to administering amphetamine orally rather than intravenously. Most importantly, the oral route should result in fewer side effects, which is critical for subject retention and safety. In addition, the duration between oral administration and radioligand injection allows assessment of neuropsychological processes for determination of possible relationships between regional amphetamine-induced dopamine release and changes in cognition and mood.

In this study we also examined the relationship between changes in dopamine and cognitive function as previous studies have yield promising findings (for a review see Cropley et al 2006a), including the study by Riccardi et al (2006) using $[^{18}\text{F}]$fallypride. Despite examining similar cognitive functions as did Riccardi et al (2006), we did not replicate their finding of significant correlations between amphetamine-induced dopamine release and change in measures of attention and speed of cognitive processing. However, we did observe significant positive correlations (corrected for multiple measures and regions with the false discovery rate (Benjamini and Hochberg
between amphetamine-induced dopamine release in the thalamus and substantia nigra and change in the controlled oral word association test, a test of phonological verbal fluency and executive function. Verbal fluency involves activation of prefrontal, left temporal, anterior cingulate as well as thalamic regions (Frith et al 1995) and is associated with dopamine function in striatum and frontal cortex (Lawrence et al 1998; Rinne et al 2000). As the thalamus forms part of the fronto-striato-thalamic neuronal circuitry (Alexander et al 1990), such a relationship with thalamic dopamine release is plausible, whereas in the substantia nigra the relationship may reflect a correlation with the overall activity of dopamine neurons, which originate in the substantia nigra and ventral tegmental area. Nevertheless, these regional correlations should be interpreted with caution as the influence of practice on cognitive change cannot be determined. The lack of correlations between regional dopamine release and other cognitive measures may be related to insufficient power due to the small sample size and inter-subject variability in amphetamine-induced dopamine release and cognitive change.

In addition, we did not detect any relationships between regional amphetamine-induced dopamine release and subjective mood, which is contrary to Drevets et al (2001) but consistent with Riccardi et al (2006), and, as discussed previously (Riccardi et al 2006), may be related to the different routes of amphetamine administration. Compared to intravenous administration, oral administration of methylphenidate - a drug which also increases synaptic dopamine concentration, produced significantly less positive reinforcing effects, and showed no correlation with striatal dopamine release, although such a correlation was seen following intravenous administration (Volkow et al 2004). Likewise, our results are consistent with the lesser euphorogenic effects of oral versus intravenous administration.

Although the sample size was small (n = 6), in contrast to Riccardi et al (2008), we found no effect of AMPT-induced dopamine depletion on $^{18}$Ffallypride binding in both striatal and extrastriatal regions in our sample of healthy volunteers. In the Riccardi study, 71.4 mg/kg AMPT over 26 h resulted in significantly increased $^{18}$Ffallypride binding (9–13%) in the caudate, putamen, ventral striatum and substantia nigra. In comparison, the effect of a slightly lower dose of AMPT on $^{18}$Ffallypride binding in the current study was variable, showing trends of paradoxical decreases in binding or no change, and large inter-subject variability. Movement of head was not a cause of the
paradoxical decreases because no subject showed significant akathisia, and the PET images were corrected for movement. Our findings also differ from those of previous studies reporting AMPT-induced increases of D2 radioligand binding in the striatum using $^{[123]}$IIBZM (+28%) (Laruelle et al 1997a) and $^{[11]}$Craclopride (+13−18.5%) (Verhoeff et al 2002; Verhoeff et al 2001), and in the temporal cortex with $^{[123]}$Iepidepride (+13%) (Fujita et al 2000).

The reasons for this discrepancy between our results and those from prior studies are unclear but are probably not related to the dose of AMPT. For example, the resulting steady-state levels of AMPT in plasma in our study (20 ± 4.3 µg/mL) were similar to those of Laruelle et al (1997a) and Fujita et al (2000). On the other hand, although plasma AMPT levels in the current study were comparable to those in previous studies, our subjects showed relatively small subjective and objective AMPT effects, at least in comparison to a previous AMPT study carried out by the same group (Fujita et al 2000). In that study, subjects experienced strong AMPT effects, indicated by two withdrawals before radioligand infusion due to akathisia and anxiety, and a greater necessity for treatment of side effects. In comparison, none of the eight subjects in the current study withdrew before starting the PET scan. This apparently weaker AMPT effect in our subjects may have been a result of insufficient central dopamine depletion, despite comparable peripheral AMPT plasma levels to other studies.

A main difference between the current study and previous AMPT radioligand binding studies was that we administered a single oral dose of amphetamine before AMPT administration. Whether this had any effect on subsequent D2 receptor measurement or on the integrity of the dopamine system is unclear. As the elimination half-life of amphetamine in adults is approximately 13−14 h (Martinsson et al 2003), there would be no residual amphetamine one week after its administration. The shortest interval between amphetamine and AMPT scans was 2 weeks. However, another possibility is that amphetamine exposure altered the sensitivity of the dopamine system, as was recently shown in a study of healthy males (Boileau et al 2006). Specifically, that study reported increased dopamine release in the striatum two weeks and 1 year following three single doses of oral amphetamine, relative to the initial amphetamine dose (i.e. sensitization). Nevertheless, a similar sensitization-like change in dopamine transmission in our subjects would have been unlikely after only one single d-
amphetamine exposure. Rather, the effect of a single oral dose of d-amphetamine on \(^{18}\text{F}\)fallypride binding likely not have exceeded 24 hours, as was shown previously with \(^{11}\text{C}\)raclopride (Cardenas et al 2004).

One limitation in the current and previous studies using \(^{18}\text{F}\)fallypride (Riccardi et al 2008; Riccardi et al 2006) is using cerebellum as the reference region. If specific binding exists in cerebellum that is affected by dopamine levels as shown for \(^{11}\text{C}\)FLB 457 (Asselin et al 2007; Montgomery et al 2007), drug-induced changes in \(^{18}\text{F}\)fallypride binding would have been underestimated. However, changes of \(^{18}\text{F}\)fallypride binding in cerebellum were not detected in monkey with amphetamine administration (Slifstein et al 2004b). Therefore, using a reference tissue model in the fallypride studies is unlikely to have caused underestimation in drug-induced changes. Another limitation is that the four PET, test, retest, amphetamine, and AMPT scans were performed in this fixed order. Although it is preferable to apply a randomized design, particularly to study neuropsychological effects, scans were performed in the fixed order because only limited information was available on prolonged effects of amphetamine and AMPT administration and a primary goal of the current study was to examine whether dopamine levels affect \(^{18}\text{F}\)fallypride binding. The length of residual effects of amphetamine and AMPT must be carefully studied before applying a randomized design.

In summary, the current study demonstrates good reproducibility of \(^{18}\text{F}\)fallypride measurements, and confirms the feasibility of measuring amphetamine-induced dopamine release in striatal and most extrastriatal regions using oral amphetamine in healthy subjects. However, contrary to recent observations, results suggest that \(^{18}\text{F}\)fallypride with AMPT treatment may be unreliable for estimating tonic or baseline dopamine levels in humans.
Chapter Six

6 Study 2: Pre- and post-synaptic dopamine imaging and its relation with frontostral cognitive function in Parkinson disease: PET studies with $[^{11}C]$NNC 112 and $[^{18}F]$FDOPA

6.1 INTRODUCTION

In the previous chapter, an experimental PET study was presented that utilized a pharmacological manipulation of the dopamine system in order to assess changes in striatal and extrastriatal dopamine markers in healthy subjects. Specifically, administration of amphetamine and AMPT was used to estimate intra-synaptic changes in phasic and tonic dopamine release respectively, via assessment of changes in $D_2$ receptor availability with $[^{18}F]$fallypride. In contrast to Study 1, which measured dopaminergic indices following dopamine alteration by means of a pharmacological intervention, Study 2 measured dopaminergic targets in a clinical population with an underlying deficit in dopamine transmission. That is, changes in dopamine markers, in comparison to normal controls, were assessed in Parkinson disease subjects who had a pathological, rather than pharmacological, alteration of the dopamine system. The effect of chronic dopaminergic alteration on both pre-synaptic (i.e. dopamine synthesis) and post-synaptic (i.e. $D_1$ receptor) targets were examined in striatal, as well as extrastriatal regions. Associations between these dopamine markers and higher cognitive functions proposed to underlie the “dorsal” frontostral circuits, which, in turn, are proposed to be severely affected in PD (refer to chapters 2 and 4, as well as below) were explored.

As described in Chapter 2, cognitive impairment is frequently observed in patients with PD, most commonly in tests of executive functioning such as working memory, planning, strategies, attentional set-shifting and concept formation (for review see Cools 2006). Alteration of the neuronal loops connecting the frontal cortex, thalamus, and basal ganglia (commonly termed frontostral circuitry) are suggested to play a role in the executive dysfunction of PD (Owen 2004). This notion is largely based on the
concept of basal ganglia organization, of which frontostriatal circuits are structurally and functionally segregated into “motor”, “limbic” and “associative” (including prefrontal) domains (Alexander et al 1990; Alexander et al 1986) (see Chapter 1 section 1.2.3 for review).

The neurochemical basis of frontostriatal and cognitive dysfunction in PD is hypothesized to be linked predominately to dopaminergic dysfunction within neural networks linking dorsal striatum (i.e. dorsolateral putamen and dorsal caudate nucleus) to dorsolateral prefrontal cortex (Cools 2006; Owen 2004). In PD, tests sensitive to dorsal frontostriatal dysfunction (so-called executive processes) such as planning and set-shifting were impaired following L-dopa withdrawal (Cools et al 2003; Hayes et al 1998; Lange et al 1992) and improved with L-dopa treatment (Bowen et al 1975; Lange et al 1993), suggesting a primarily dopaminergic substrate. Further, a cerebral blood flow study in PD patients demonstrated dopaminergic modulation of frontostriatal networks during planning (Cools et al 2002). While together these studies provide strong evidence linking dopamine with frontostriatal executive processes, the findings do not directly address the locus of the molecular pathology and its relationship to the cognitive dysfunction.

Positron emission tomography allows direct \textit{in vivo} assessment of pre- and postsynaptic dopaminergic function in PD. Presynaptic markers of dopamine neurons include $[^{18}\text{F}]$FDOPA and dopamine transporter ligands, which are consistently lower in the striatum of PD patients (Heiss and Hilker 2004) (for review see Chapter 1 section 1.3.1). Several PET and SPECT studies have correlated striatal, especially caudate nucleus, dopamine loss with cognitive disturbance in PD (Bruck et al 2001; Holthoff-Detto et al 1997; Marie et al 1999; Muller et al 2000; Rinne et al 2000) (refer to Chapter 2 for overview). Recently, $[^{18}\text{F}]$FDOPA has also been assessed in the cortex of PD patients and was reduced in the frontal cortex (Rinne et al 2000) and anterior cingulate (Ito et al 2002). Reductions of frontal cortical $[^{18}\text{F}]$FDOPA uptake in PD was also associated with impairments in working memory, verbal fluency and suppressed attention (Bruck et al 2005; Rinne et al 2000). These findings indicate involvement of both striatal and cortical dopamine depletion in the executive impairment of PD, although the precise relationship with frontostriatal tasks such as planning and set-shifting remains unclear.
As reviewed in Chapter 2, work from experimental animals suggests a critical role for post-synaptic dopamine D$_1$ receptors within the prefrontal cortex in modulating certain executive processes, particularly working memory but also cognitive flexibility or set-shifting processes (Arnsten 1997; Arnsten 1998; Floresco and Magyar 2006; Sawaguchi and Goldman-Rakic 1991). In humans, examination of D$_1$ receptors in executive processes has been limited owing to the lack of selective D$_1$ compounds for human use. Whether D$_1$ receptors are altered in PD is largely unknown. Two PET studies have not shown changes in D$_1$ receptor density in striatum and orbitofrontal cortex of PD patients (Ouchi et al 1999a; Shinotoh et al 1993), but both studies used the D$_1$ ligand $[^{11}C]$SCH 23390 ($K_D = \sim 0.4 – 0.14$ nM), which has low specific-to-nonspecific ratios (Karlsson et al 1997). The D$_1$ ligand $[^{11}C]$NNC 112 also displays high affinity for the D$_1$ receptor ($K_D = 0.18$ nM) but shows greater specific to non-specific binding than $[^{11}C]$SCH 23390 (Halldin et al 1998). Increases in $[^{11}C]$NNC 112 binding in the prefrontal cortex were associated with impairments of working memory in schizophrenia (Abi-Dargham et al 2002). Whether D$_1$ receptors are associated with frontostriatal cognitive processes in PD is not known.

The purpose of the current study was to investigate the relationship between pre- and postsynaptic dopamine markers within the frontostriatal circuitry and executive function in PD. Specifically, the role of striatal and cortical dopamine function on frontostriatal executive processes in non-demented PD were assessed with $[^{18}F]$FDOPA (a measure of pre-synaptic dopamine synthesis), $[^{11}C]$NNC 112 (a marker of post-synaptic D$_1$ receptors), and two frontostriatal cognitive tests (the Stockings of Cambridge planning task and the Wisconsin card sorting test). This study has been accepted for publication in Psychiatry Research: Neuroimaging (see Appendix 3 for reprint of uncorrected proof).

### 6.2 MATERIALS AND METHODS

#### 6.2.1 Subjects

Fifteen non-demented, moderately impaired patients with idiopathic Parkinson disease (9 males, 6 females) and 14 age-matched healthy volunteers (8 males, 6 females) participated in the study (Table 6-1). Patients met the diagnosis of idiopathic PD based
upon the presence of at least two of the four cardinal symptoms (tremor, bradykinesia, rigidity and instability) as well as a positive response to dopamine agents. Patients were non-smokers and were free of current medical and neurological disorder not related to Parkinson disease. No patient met current criteria for major depression disorder, as assessed with the Structured Clinical Interview for DSM-IV Disorders. Controls were non-smokers, medically and neurologically healthy and were free of current psychiatric illness according to DSM-IV Axis I criteria. Both PD and control participants were excluded if they showed more than moderate hypertension (defined as >160/95) at the initial screening. Participants (both patients and controls) were non-demented and were excluded if they scored <24 on the Mini-Mental State Exam (Folstein et al 1975). In an elderly population, a cut-off of 24 gives high sensitivity and specificity (Spreen and Stauss 1991). All but one patient was being treated for PD with L-dopa (e.g. Sinemet® or Stalevo®), 7 with the D2/D3 agonist pramipexole and one with amantadine. Each patient had their medication stopped at least 12 h before the [18F]FDOPA PET scan and were clinically assessed in a stable drug-free state using part III (motor examination) of the Unified Parkinson’s Disease Rating Scale on the morning of the PET scan. Because the off medication state may cause a fluctuation in blood pressure, for safety precautions patients stayed overnight at the NIH Clinical Centre the night before the [18F]FDOPA scan. After admission, blood pressure was monitored approximately every four hours while the patient was awake. Subjects were discontinued from participating in the [18F]FDOPA scan if blood pressure increased over 180/100 for more than 15-30 min. Likewise, on baseline evaluation on each of the PET days, the scan was discontinued if blood pressure >180/100 continuously for 15-30. Patients were at clinical stage 1.5 – 4 using the Hoehn and Yahr (1967) classification system. The Radiation Safety Committee of the National Institutes of Health and the Institutional Review Board of the National Institute of Mental Health approved the study. All subjects provided written informed consent to participate in this study.

6.2.2 Radiopharmaceutical preparation and dosimetry

The (+)-desmethyl-NNC 112 (1.0 mg per radiolabeling) was obtained from Professor Christer Halldin of the Karolinska Institutet. [11C]NNC 112 was synthesized from [11C]methyl iodide (Halldin et al 1998) via a captive solvent method using a
commercially available radiochemistry “loop” module. The radiochemical purities of all $[^{11}\text{C}]$NNC 112 batches was greater than 99%. $[^{18}\text{F}]$FDOPA was produced using the method of Adam and Jivan (1998) with slight modifications to the purification steps. The radiochemical purities of all $[^{18}\text{F}]$FDOPA batches was greater than 90%. Because there were no previous radiation-absorbed dose estimates of $[^{11}\text{C}]$NNC 112 in human subjects, whole body imaging scans of $[^{11}\text{C}]$NNC 112 were performed in healthy volunteers prior to initiation of this study. These scans demonstrated that $[^{11}\text{C}]$NNC 112 displays a favorable radiation dose profile in humans, showing an effective dose of 5.7 μSv/MBq. $[^{11}\text{C}]$NNC 112 radiation dosimetry estimates is published in the Journal of Nuclear Medicine (Cropley et al 2006b) (see Appendix 4 for reprint of article).

6.2.3 Scanning protocol

PET scans were performed on a GE Advance tomograph. After the transmission scan, dynamic emission scans were acquired following an intravenous bolus injection of 379 – 601 MBq of $[^{18}\text{F}]$FDOPA and 391 – 766 MBq of $[^{11}\text{C}]$NNC 112 for a total scan time of 90 min (6 × 30 s, 3 × 1 min, 2 × 2 min, 16 × 5 min). Scans were reconstructed with the filtered-back projection algorithm which resulted in a final image resolution of 7.5 mm FWHM. For the $[^{18}\text{F}]$FDOPA scan, all subjects received an oral dose of 200 mg carbidopa, a peripheral inhibitor of AADC, 1 h before scanning. Administration of carbidopa is customary in $[^{18}\text{F}]$FDOPA PET imaging to increase the availability of $[^{18}\text{F}]$FDOPA in the brain. To minimize inter-subject variability in carbidopa absorption, subjects were prohibited to eat food one hour before and after carbidopa administration. As mentioned previously, PD patients were in an off-medication state for the $[^{18}\text{F}]$FDOPA scan. Because FDOPA is an analogue of L-dopa (refer to Chapter 1), withdrawal of L-dopa containing medications is necessary and a standard procedure in $[^{18}\text{F}]$FDOPA imaging of PD patients to prevent L-dopa from interfering with the uptake of $[^{18}\text{F}]$FDOPA. Administration of PD patients’ L-dopa medication was resumed after termination of the $[^{18}\text{F}]$FDOPA PET scan. One PD patient did not complete the $[^{18}\text{F}]$FDOPA scan due to transient high blood pressure (see above subject criteria) after discontinuing L-dopa medication. For the $[^{11}\text{C}]$NNC 112 scans, PD patients continued their normal medication regime. Approximately half of the subjects in each group underwent the $[^{18}\text{F}]$FDOPA scan first. The average interval between $[^{18}\text{F}]$FDOPA and
[11C]NNC 112 scans was 18 days. All subjects received an MRI scan for coregistration and segmentation purposes, with IR-FGRE, T2 and FLAIR images being obtained.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Parkinson disease</th>
<th>Controls</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>62.1±9.2</td>
<td>61.6±8.0</td>
<td>0.16</td>
<td>0.878</td>
</tr>
<tr>
<td>Education</td>
<td>15.3±2.8</td>
<td>16.8±3.1</td>
<td>1.40</td>
<td>0.174</td>
</tr>
<tr>
<td>Mini-Mental State Exam(^a)</td>
<td>29.1±1.0</td>
<td>29.3±1.0</td>
<td>94.5(^b)</td>
<td>0.646</td>
</tr>
<tr>
<td>Beck Depression Inventory(^a)</td>
<td>1.8±2.8</td>
<td>0.1±0.3</td>
<td>46.5(^b)</td>
<td>0.003</td>
</tr>
<tr>
<td>Dementia Rating Scale Total</td>
<td>139±5.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Motor Unified Parkinson Disease Rating Scale</td>
<td>41.9±10.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hoehn &amp; Yahr</td>
<td>3.0±0.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Disease duration (yrs)</td>
<td>11.7±5.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>[11C]NNC Injected Activity (MBq)</td>
<td>704±111</td>
<td>651±103</td>
<td>1.32</td>
<td>0.199</td>
</tr>
<tr>
<td>[11C]NNC Specific Activity (GBq/µmol)</td>
<td>47.6±12.3</td>
<td>63.5±49.6</td>
<td>1.16</td>
<td>0.264</td>
</tr>
<tr>
<td>[18F]FDOPA Injected Activity (MBq)(^a)</td>
<td>574±15.5</td>
<td>543±49.3</td>
<td>2.22</td>
<td>0.035</td>
</tr>
<tr>
<td>[18F]FDOPA Specific Activity (GBq/µmol)(^a)</td>
<td>35.3±5.3</td>
<td>32.7±6.3</td>
<td>1.18</td>
<td>0.250</td>
</tr>
<tr>
<td>Wisconsin Card Sorting Test perseverative responses(^a)</td>
<td>24.5±17.9</td>
<td>17.2±15.0</td>
<td>70.0(^b)</td>
<td>0.130</td>
</tr>
<tr>
<td>Wisconsin Card Sorting Test categories achieved(^a)</td>
<td>2.9±2.5</td>
<td>4.6±1.9</td>
<td>66.0(^b)</td>
<td>0.080</td>
</tr>
<tr>
<td>Stockings of Cambridge Initial think time 5 moves(^a) (s)</td>
<td>16.7±19.0</td>
<td>13.7±6.2</td>
<td>72.0(^b)</td>
<td>0.375</td>
</tr>
<tr>
<td>Stockings of Cambridge perfect solutions(^a)</td>
<td>8.4±2.1</td>
<td>8.0±2.1</td>
<td>83.0(^b)</td>
<td>0.705</td>
</tr>
</tbody>
</table>

Data are mean ± standard deviation. Group comparisons performed with an independent samples t-test. \(^a\)Group comparison performed with Mann-Whitney U non-parametric test. \(^b\)Mann-Whitney U value. P-values are two-tailed.
6.2.4 Neuropsychological tests

All subjects were administered the Mini-Mental State Exam (Folstein et al 1975). We included patients with a score of 24 or greater. The Dementia Rating Scale-2 (Jurica et al 2001), with a cut-off score of $\geq 123$, was additionally administered to PD patients to evaluate overall cognitive performance and rule out dementia. Depressive symptoms were evaluated with the Beck Depression Inventory fast screen (Beck et al 2000). The fast screen does not include somatic items that are common to certain medical conditions, substances or age and thus more appropriate for patients with PD and an elderly population. To examine frontostriatal functioning, subjects were administered a computerized version of the Wisconsin Card Sorting Test (Heaton and Goldin 2003) and the Stockings of Cambridge task (Cambridge Cognition, UK), which is a computerized form of the Tower of London test of planning (Shallice 1982). These or similar tests are impaired in some non-demented, medicated PD patients (Owen et al 1992; Taylor et al 1986) and are also sensitive to dopaminergic manipulation (Cropley et al 2006a). The Wisconsin test involves subjects to match multi-dimensional test cards to reference cards according to the color, shape or number of the card stimuli. Subjects are not told how to match the cards but must acquire the correct rule of classification. After a fixed number of correct matches the classification rule changes, requiring subjects to shift their response set to a new stimulus dimension. Performance on the Wisconsin test was based on the number of categories achieved (0–6) and the number of perseverative responses. As detailed in the previous chapter, the Stockings of Cambridge task consists of two arrangements of three colored balls, one in the top half of the screen and the other in the bottom half. Subjects are required to rearrange the balls in the bottom display to match the “goal” arrangement in the top display in the minimal number of moves. For this task, initial thinking time for the most difficult (5 move) problems and number of perfect solutions (across all levels of difficulty) were used. PD patients performed the neuropsychological tasks in an “on-state,” defined as 30 – 60 min after medication and the patient’s subjective feeling of being in an on-state. Patients remained on their dopamine replacement therapy to minimize the effect of motor impairment on test scores and because deficits in these tasks have been observed in medicated patients. One PD patient and one healthy control did not complete the Stockings of Cambridge task due to time constraints and technical problems, respectively.
6.2.5 Statistical analysis

Group comparisons (two-tailed) of demographic, clinical and PET variables were performed using independent sample \( t \)-test for parametric data and Mann-Whitney U test for non-parametric data. Non-parametric variables were determined by the Shapiro-Wilk normality test. Correlations between neuropsychological measures and frontostriatal regions were performed with Spearman’s rank correlation or Pearson’s correlation coefficient as appropriate. Multiple comparisons were controlled for with the false discovery rate procedure (Benjamini and Hochberg 1995), previously described in the general methods chapter. To reiterate, the FDR approach orders the \( P \) values \( (P_{(i)}) \) from highest to lowest and sequentially compares each \( P_{(i)} \) with \( 0.05i/m \), with \( m \) being the number of comparisons made. If a \( P \) value satisfies the constraint, all hypotheses below this \( P \) value are also rejected (see chapter 4 for further description of the FDR procedure). All statistical analyses, except voxel-based, were performed using SPSS for Windows.

6.2.6 Image analysis

*Preprocessing and parametric imaging*

To correct for head movement during the scan, PET frames of both \[^{11}\text{C}]\text{NNC 112} and \[^{18}\text{F}]\text{FDOPA} were realigned to a standard frame using the FLIRT algorithm (Jenkinson and Smith 2001) and MRI (IR, T2 and FLAIR) was coregistered to an average image of initial frames of each of \[^{11}\text{C}]\text{NNC 112} and \[^{18}\text{F}]\text{FDOPA} using SPM2. Parametric images of PET data were calculated using PMOD 2.65. For \[^{18}\text{F}]\text{FDOPA} PET scans, parametric images in which each pixel represents the influx constant, \( K_i \) (min\(^{-1}\)) of \[^{18}\text{F}]\text{FDOPA} were calculated with the Patlak graphical analysis (Patlak and Blasberg 1985). For each subject, putamen (target) and occipital cortex (reference) volumes of interest were obtained in the Montreal Neurological Institute stereotaxic space. A Patlak plot of the time-activity curve in putamen was used to determine the start time of the linear segment (\( t^* \)) of the graph. This same \( t^* \) was used for pixel-wise calculations of \( K_i \) in all target regions. The slope of the linear segment equals the influx constant \( K_i \), and represents the uptake rate constant of \[^{18}\text{F}]\text{FDOPA}. For \[^{11}\text{C}]\text{NNC 112} scans, parametric images of binding potential (\( BP_{ND} \)) and \( K_i/K_{i'} \) relative ligand delivery (\( R_i \)), were generated using the Multilinear Reference Tissue Model 2 (Ichise et al 2003).
$BP_{\text{ND}}$ refers to the ratio at equilibrium of specifically bound radioligand to that of nondisplaceable radioligand in tissue (see Innis et al 2007), while $R_1$ is a measure of radioligand delivery to tissue relative to the reference region. Putamen and cerebellum volumes of interest (obtained in Montreal Neurological Institute space) were used as receptor-rich and reference regions respectively. All data points were used in the fitting for $[^{11}\text{C}]\text{NNC 112}$, since Logan plots are fairly linear from early time points in a previous study (Abi-Dargham et al 2000a). Parametric images of $[^{18}\text{F}]\text{FDOPA }K_i$ were coregistered to $[^{11}\text{C}]\text{NNC 112}$ space and subsequently both $[^{18}\text{F}]\text{FDOPA}$ and $[^{11}\text{C}]\text{NNC 112}$ parametric images were spatially normalized to a custom template of the study sample created from 15 PD and 13 control subjects’ $[^{11}\text{C}]\text{NNC 112 }R_1$ parametric images.

**Partial volume correction**

Because the thickness of cortical gray matter is only a few millimeters, PET data are a mixture of gray and white matter. We applied partial volume correction to $[^{18}\text{F}]\text{FDOPA}$ PET to minimize the white matter influence on the gray matter signal. $[^{11}\text{C}]\text{NNC 112}$ data also underwent partial volume correction for purposes of comparison. Partial volume correction was performed using three segments (gray matter, white matter, and cerebrospinal fluid) of MRIs (Muller-Gartner et al 1992) created from IR, T2 and FLAIR images using SPM2 and coregistered to PET using the previously determined PET/MRI transformation parameters. Binary mask images for gray and white matter were smoothed with a 7.5 mm Gaussian filter. Gray matter pixels were corrected for spill-out of activity and for spill-in of activity from white matter. To do this, white matter activity was subtracted from the uncorrected image and divided by the smoothed gray matter image. Pure white matter activity was estimated by extrapolating with linear regression the activity values of pixels with a white matter membership greater than 99% (Giovacchini et al 2004). To eliminate noisy voxels, the resulting corrected image was thresholded so there was at least a 20% probability for the pixel to belong to gray matter. PMOD was used to perform partial volume correction. Parametric images of corrected $[^{18}\text{F}]\text{FDOPA }K_i$ and $[^{11}\text{C}]\text{NNC 112 }BP_{\text{ND}}$ were created as described above.

**Region of interest analysis**
Region of interest within the frontostriatal circuitry were applied to $K_i$ and $BP_{ND}$ parametric images normalized to the study sample template. Striatal regions were defined bilaterally on the caudate nucleus and putamen of a mean image of the study sample’s spatially normalized MRI. Extrastriatal regions were taken from the anatomical labeling template (Tzourio-Mazoyer et al 2002) and were defined on the superior, middle and inferior (triangular) lateral frontal gyri and thalamus. Independent sample $t$-tests were performed to compare average $K_i$ and $BP_{ND}$ of patients and controls. Correlations between neuropsychological variables and $K_i$ and $BP_{ND}$ were assessed with Spearman’s or Pearson’s correlation.

**SPM analysis**

Voxel-based statistical analysis of parametric $K_i$ and $BP_{ND}$ images were performed using SPM2. An isotropic 10-mm Gaussian kernel was used to smooth normalized parametric images. As $K_i$ and $BP_{ND}$ values are quantitative, all SPM analyses were performed without global normalization. Between-group comparisons of $K_i$ and $BP_{ND}$ at voxel-level were performed using a two-sample $t$-test. Analyses testing the correlation between neuropsychological score and $K_i$ or $BP_{ND}$ values were performed with a regression analysis. False discovery rate of $P$ less than 0.05 (voxel-level) was considered significant. Because [^{18}F]FDOPA data in extrastriatal areas is contaminated by white matter (see below), $K_i$ analyses were done using small-volume correction, i.e. restricted to the striatum. The striatal mask was created from the average [^{18}F]FDOPA parametric image of healthy subjects. Covariate analyses of $BP_{ND}$ were made on whole brain parametric images to explore possible correlations outside the frontostriatal network. Voxel-wise analysis was not performed on partial volume corrected data.

### 6.3 RESULTS

#### 6.3.1 Demographic and neuropsychological data

PD patients did not significantly differ from controls in age, education or Mini-Mental State Examination score (Table 6-1). Although PD patients reported significantly more symptoms of depression on the Beck Depression Inventory fast screen, the mean score indicated mild depressive symptoms, and no patients were clinically depressed. Furthermore, there were no significant correlations (Spearman’s, two-tailed) between
the Beck Depression Inventory and PET and cognitive measures in PD patients. Patients did not significantly differ in performance from controls on any of the neuropsychological measures, although they did not complete as many Wisconsin categories as controls (Table 6-1). Cognitive performance of PD patients was variable, consisting of both high and low performing individuals. Six PD patients (40%) were identified as being cognitively impaired (defined as falling within the 5th percentile of the cognitive test based on normative data) on at least one neuropsychological measure.

6.3.2 \[^{18}\text{F}]\text{FDOPA uptake}\n
Striatal \[^{18}\text{F}]\text{FDOPA uptake}\n
The mean $K_i$ in putamen and caudate was significantly decreased in PD patients compared to controls (Table 6-2) with both SPM and region of interest analysis. In patients, the putamen showed lower $K_i$ values than the caudate nucleus. \[^{18}\text{F}]\text{FDOPA}\ influx constant was reduced in PD patients by 70% in the putamen (SD = 0.04, range: 64 – 80%) and by 36% in the caudate nucleus (SD = 0.11, range: 19 – 56%). PD patients showed lateralized differences in striatal $K_i$, which was significantly lower (Paired $t$-test, $t=3.7$, $df=13$, $P=0.003$,) in striata (putamen and caudate) contralateral to the side of the body with the initial presentation of symptoms in all, except for two, patients.

<table>
<thead>
<tr>
<th>Region</th>
<th>Parkinson disease</th>
<th>Controls</th>
<th>$t$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caudate</td>
<td>7.1±1.2</td>
<td>11.1±1.1</td>
<td>9.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Putamen</td>
<td>3.4±0.5</td>
<td>11.3±1.3</td>
<td>21.5</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Mean ± SD \[^{18}\text{F}]\text{FDOPA}\ influx constant ($K_i \times 10^{-3}$ min$^{-1}$)

Group comparison performed with an Independent sample $t$-test.

6.3.3 \[^{18}\text{F}]\text{FDOPA uptake in extrastriatal regions}\n
\[^{18}\text{F}]\text{FDOPA}\ $K_i$ values in extrastriatal regions were considerably lower than in striatum. $K_i$ values were unexpectedly higher in cerebral white matter than adjacent gray matter regions (Figure 6-1). Values were approximately 2–3 fold higher in white matter than
frontal gyrus regions. Unlike the asymmetry in striata, $K_i$ in frontal cortex of patients was not related with the side of body showing initial symptoms.

![Figure 6-1](image)

**Figure 6-1.** (a) Mean parametric image of $[^{18}\text{F}]$FDOPA $K_i$ of healthy subjects superimposed onto MRI template. $K_i$ values are greater in cortical white matter than adjacent gray matter regions (b). The kinetics of white matter (▲) uptake and washout from a representative healthy subject is significantly different from reference region occipital cortex (■) (c).

### 6.3.4 Partial volume correction of $[^{18}\text{F}]$FDOPA PET

Minimizing the influence of white matter data by partial volume correction decreased $K_i$ and increased inter-subject variability in all frontal cortical regions studied. Partial volume correction decreased $K_i$ by approximately 45% and increased COV (SD/mean) by 45 to 115% in PD patients (Table 6-3), and decreased $K_i$ by a similar amount (42%) and increased COV by 12 to 55% in controls. Partial volume correction typically increases gray matter PET values, because the signal or variable of interest is typically higher in gray than white matter. Partial volume correction decreased gray matter $K_i$ values because of greater $K_i$ in white matter. Because of unreasonably high $K_i$ values in white matter and small and variable $K_i$ values with partial volume correction, $[^{18}\text{F}]$FDOPA analyses were only conducted in the striatum.
Table 6-3 Effect of partial volume correction on frontal [18F]FDOPA influx constants in Parkinson disease patients

<table>
<thead>
<tr>
<th>Region</th>
<th>$K_i$ no PVC</th>
<th>COV</th>
<th>$K_i$ PVC</th>
<th>COV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superior frontal gyrus</td>
<td>1.00±0.27</td>
<td>0.27</td>
<td>0.57±0.33</td>
<td>0.58</td>
</tr>
<tr>
<td>Middle frontal gyrus</td>
<td>0.88±0.23</td>
<td>0.26</td>
<td>0.54±0.27</td>
<td>0.50</td>
</tr>
<tr>
<td>Inferior frontal gyrus</td>
<td>1.30±0.26</td>
<td>0.20</td>
<td>0.70±0.20</td>
<td>0.29</td>
</tr>
</tbody>
</table>

Mean ± SD [18F]FDOPA influx constant ($K_i \times 10^{-3} \text{min}^{-1}$) in PD patients before and after partial volume correction (PVC). COV = SD/mean

6.3.5 [11C]NNC 112 binding

Between-group SPM and region of interest analysis showed no significant differences or trends in regional [11C]NNC 112 $BP_{ND}$ between PD patients and controls (Table 6-4). Patients showed only a weak trend of lower $BP_{ND}$ values with the smallest false discovery rate-corrected $P$ value of 0.16 in the right median cingulate. Striatal $BP_{ND}$ was approximately 7-fold higher than that in frontal regions, which is consistent with the known distribution of D1 receptors in brain (De Keyser et al 1988; Hall et al 1994). A mean parametric image of [11C]NNC 112 $BP_{ND}$ in healthy subjects illustrates markedly higher $BP_{ND}$ in striatal than extrastriatal regions (Figure 6-2). Patients did not show marked asymmetry of $BP_{ND}$ in striatum. $BP_{ND}$ values in striata and frontal cortex of patients was not related with the side of body showing initial symptoms. Partial volume correction of [11C]NNC 112 increased $BP_{ND}$ values 2.5 fold and decreased inter-subject variability (COV) by 50%. [11C]NNC 112 $BP_{ND}$ values were markedly lower across brain regions in our subjects compared to control subjects of a previous PET study using [11C]NNC 112 (Abi-Dargham et al 2002).
Table 6-4. $[^{11}C]$NNC 112 binding potential from region of interest analysis

<table>
<thead>
<tr>
<th>Region</th>
<th>Parkinson disease</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caudate</td>
<td>1.98±0.38</td>
<td>2.03±0.36</td>
</tr>
<tr>
<td>Putamen</td>
<td>2.35±0.39</td>
<td>2.18±0.38</td>
</tr>
<tr>
<td>Thalamus</td>
<td>0.36±0.99</td>
<td>0.35±0.11</td>
</tr>
<tr>
<td>Superior frontal gyrus</td>
<td>0.26±0.10</td>
<td>0.29±0.09</td>
</tr>
<tr>
<td>Middle frontal gyrus</td>
<td>0.32±0.11</td>
<td>0.34±0.08</td>
</tr>
<tr>
<td>Inferior frontal gyrus</td>
<td>0.33±0.11</td>
<td>0.37±0.10</td>
</tr>
</tbody>
</table>

No significant differences in any region. ($P$-values 0.26 – 0.76)

Figure 6-2. Mean parametric image of $[^{11}C]$NNC 112 $BP_{ND}$ of healthy subjects. Binding potential values are clearly higher in striatum compared to extrastriatal areas such as frontal cortex, which is consistent with the known distribution of $D_1$ receptors in human brain. In contrast to $[^{18}F]$FDOPA, $BP_{ND}$ values are greater in gray than white matter regions.

6.3.6 Correlational analyses

For $[^{11}C]$NNC 112, there were no significant correlations between neurocognitive scores and $BP_{ND}$ in PD and controls with region of interest and SPM analysis. For $D_1$ receptors, the maximum correlation was observed between $BP_{ND}$ in the caudate of healthy controls and Stockings of Cambridge perfect solutions (Pearson’s $r = 0.67$, $P = 0.013$), although this did not survive multiple comparison correction. For $[^{18}F]$FDOPA,
correlations were restricted to only the striatum. With region of interest analysis, PD patients showed a significant positive correlation between $K_i$ in the putamen and number of categories achieved on the Wisconsin card sorting test (Spearman’s $\rho = 0.69$, $P = 0.006$) (Figure 6-3). SPM with small volume correction was applied to detect correlations in striatal subdivisions, which would have been missed in the region analysis. The SPM analysis did not show significant correlations. No other correlations were found with other neuropsychological measures or in the caudate. Disease severity indices and age were not related to neuropsychological measures in PD, and partial correlations with age as the control variable produced almost identical statistical results. Region of interest and SPM analysis showed no significant correlations between striatal $K_i$ and neurocognitive scores in healthy controls.

![Figure 6-3](image)

**Figure 6-3.** Wisconsin card sorting test categories achieved score versus $[^{18}\text{F}]$FDOPA uptake ($K_i$) in the putamen of PD patients (Spearman rho = 0.69, $P = 0.006$, two-tailed).

### 6.4 DISCUSSION

In this sample of non-demented PD patients, we found no differences in dopamine D$_1$ receptor density in fronto-striatal-thalamic regions and no overall difference in frontostriatal cognitive performance. Variability in performance in PD patients on a task reliant on the integrity of the “dorsal” frontostriatal circuitry was associated with
dopamine loss in the putamen. D<sub>1</sub> receptor density did not significantly correlate with cognitive performance on frontostriatal tests. [¹⁸F]FDOPA uptake values in white matter were erroneously higher than those in gray matter, which casts significant doubt on the validity of cortical dopamine synthesis measurements with [¹⁸F]FDOPA.

6.4.1 Presynaptic dopamine synthesis and questionable measurement of cortical [¹⁸F]FDOPA

Consistent with over two decades of research, our PD patients showed reduced K<sub>i</sub> in striatum, with greater loss in the putamen than caudate nucleus. Unexpectedly however, we found higher [¹⁸F]FDOPA K<sub>i</sub> in white than adjacent gray matter, which is unreasonable since AADC is minimally present in white matter. Over a decade of published [¹⁸F]FDOPA studies using Patlak parametric modeling with a reference tissue input have not, to our knowledge, reported this phenomenon. One paper has shown an [¹⁸F]FDOPA parametric image with apparent greater K<sub>i</sub> in white matter (Nagano et al 2000), although this was not mentioned. This error in quantification of the cortical [¹⁸F]FDOPA signal was possibly caused by slower washout of radioactivity from white compared to gray matter. The reference region (occipital cortex) contains both gray and white matter, and its kinetics would be different from that of either frontal gray or white matter. Such a discrepancy with the reference region would violate an assumption of the Patlak and Blasberg (1985) model that the ratio of activity in nondisplaceable compartments in target compared to reference regions should be constant after t*. We found that the time-activity curves were markedly different between white matter and occipital cortex, which violated this assumption and caused K<sub>i</sub> values to be erroneously greater in white matter. Removal of white matter from PET images with partial volume correction to circumvent this model violation actually reduced gray matter K<sub>i</sub> and increased intersubject variability. Rather than increasing the specific signal in gray matter, as occurred with [¹¹C]NNC 112, the signal became considerably smaller and more noisy after partial volume correction, therefore making it vulnerable to statistical noise. Therefore, [¹⁸F]FDOPA K<sub>i</sub> values in extrastriatal regions are unreliable and did not undergo further between-group and correlational analyses.

Our finding of greater K<sub>i</sub> in white than adjacent gray matter places significant doubt on the validity and interpretation of cortical [¹⁸F]FDOPA with reference input models. Six
studies have reportedly measured cortical [\(^{18}\text{F}\)]FDOPA uptake in PD patients (Bruck et al 2005; Ito et al 2002; Kaasinen et al 2001; Rakshi et al 1999; Rinne et al 2000) or normal elderly controls (Nagano et al 2000) using cerebellum or occipital cortex as reference regions. Such studies have reported increases (Bruck et al 2005; Kaasinen et al 2001; Rakshi et al 1999) and decreases (Ito et al 2002; Rinne et al 2000) in \(K_i\) in cortex, which has been interpreted as reflecting either increased or decreased dopamine synthesis. Although parametric images were analyzed, most of these studies presented [\(^{18}\text{F}\)]FDOPA images by summing up data obtained during the entire scan. Please note that such images do not reflect \(K_i\) because images at early time points reflect mainly blood flow. Since early images have greater activity than late images, a large portion of the summed up images reflect merely blood flow but not the metabolism of [\(^{18}\text{F}\)]FDOPA to [\(^{18}\text{F}\)]Fluorodopamine. It is questionable whether [\(^{18}\text{F}\)]FDOPA gives a specific PET signal and is meaningful in cortex for the following reasons. 1). Comparisons of \(K_i\) in gray and white matter are unreasonable and may be due to a model violation. 2). DOPA decarboxylase activity is very low in frontal cortex of human brain tissue and activity ratio of cortex/caudate is 1% (Mackay et al 1978). Our ratio of frontal cortex/caudate \(K_i\) after partial volume correction in healthy controls was high, with a value of 4%. Previous studies without partial volume correction have shown unrealistically high cortex/caudate ratios of \(K_i\) in controls, ranging between 10 – 30% (Bruck et al 2005; Ito et al 2002; Kaasinen et al 2001; Nagano et al 2000; Rakshi et al 1999; Rinne et al 2000), suggesting that [\(^{18}\text{F}\)]FDOPA measurements are not specific to DOPA decarboxylase activity. By not applying partial volume correction, in other words, leaving greater influence from white matter data, frontal cortex/caudate \(K_i\) in the current study became 8%, which is close to the ratios reported in previous studies. And 3). Tyrosine hydroxylase, the first step dopamine-synthesizing enzyme, and AADC do not coexist in neurons in human cingulate cortex (Ikemoto et al 1999), suggesting that AADC-only neurons in at least the cingulate are not specific to dopamine. As such, the scientific community should be aware that the cortical [\(^{18}\text{F}\)]FDOPA signal has serious deficiencies.
6.4.2 Postsynaptic dopamine D₁ receptors in Parkinson disease

Lack of alteration of dopamine D₁ receptors in our PD sample is consistent with previous studies showing no regional differences in [¹¹C]SCH 23390 binding in PD (Ouchi et al 1999a; Shinotoh et al 1993). Ouchi et al (1999a) studied only early (Hoehn and Yahr stage 1 and 2) PD patients while Shinotoh et al (1993) examined a heterogeneous sample consisting of patients in Hoehn and Yahr stages 1 – 4 and disease duration of 6 months to 10 years. Our study found no changes in D₁ receptors in PD patients with moderate symptom severity using a different radioligand for measuring D₁ receptors in low-density, cortical regions. Taken together, these studies suggest that postsynaptic dopamine D₁ receptors are not altered in PD, at least in early to moderate stage patients. A problem with these ligands however, is their affinity to cortical 5-HT₂A receptors. A very recent study has shown that about 20 to 30% of cortical [¹¹C]NNC 112 uptake in humans is to 5-HT₂A receptors (Slifstein et al 2007), making [¹¹C]NNC 112 almost equivalent to [¹¹C]SCH 23390 with regard to D₁ receptor selectivity (Ekelund et al 2007). Development of more selective ligands for D₁ receptors is therefore needed to adequately assess changes in cortical D₁ receptor expression in PD.

Due to feasibility issues, PD patients remained on their normal dopaminergic medications for the [¹¹C]NNC 112 PET scan. This included L-dopa and dopamine D₂/D₃ agonists such as pramipexole and amantadine. Although these medications are not known to directly interact with D₁ receptors, it is possible that the indirect increases in extracellular dopamine generated by L-dopa may lead to changes in D₁ receptors. Such changes however, are probably unlikely. For example, no changes in D₁ receptors have been found in drug-naive PD patients (Ouchi et al 1999a; Shinotoh et al 1993) and treated (but medication withdrawn) patients (Shinotoh et al 1993), and further, no changes in [¹¹C]NNC 112 binding was shown following acute administration of the dopamine agonist, amphetamine, in monkeys (Chou et al 1999). This suggests that dopaminergic medication should have little effect on D₁ receptor PET measurement. On the other hand, long-term L-dopa exposure was observed to downregulate D₁ receptors (Turjanski et al 1997), and while no evidence of this was noted in the current study, the possibility exists that a relative D₁ receptor downregulation could result in a relative D₁ receptor normalization (see Chapter 7 for further discussion). Medication-induced interactions as a potential confound on [¹¹C]NNC 112 binding cannot be ruled out and
requires clarification by measuring $D_1$ receptor availability on and off L-dopa medication.

Binding potential of $[^{11}\text{C}]\text{NNC 112}$ was about 65 – 75% lower in our study compared to values reported by Abi-Dargham et al (2002) in their healthy cohort. This discrepancy was possibly due to differences in the methods for obtaining region of interest data and age-related decline of $D_1$ receptors in human brain (Suhara et al 1991; Wang et al 1998), as the subjects in the current study were approximately 30 years older than those in the Abi-Dargham study.

### 6.4.3 Frontostriatal cognitive function in Parkinson disease and association with pre- and postsynaptic dopamine markers

Our sample of moderately severe PD patients did not show overall frontostriatal cognitive impairment in comparison with elderly controls. Previous studies have reported impairments on the Wisconsin card sorting test (Bowen et al 1975; Brown and Marsden 1988; Canavan et al 1989; Paolo et al 1995; Taylor et al 1986) and Tower of London type planning tasks (Dubois and Pillon 1997; Owen et al 1992) in medicated, non-demented PD patients, and in patients with a moderate severity of symptoms (Brown and Marsden 1988; Owen et al 1992). Our PD patients were tested in an “on-state” relative to their medication effectiveness because some tests are affected by motor function. Thus, the administration of dopaminergic medication may have had a facilitating or normalizing effect on their executive processes and contributed to the lack of overall cognitive impairment. Testing patients both on and off dopamine medication would help determine the effect of dopamine treatment on cognitive processes.

Although PD patients showed no overall cognitive impairment they did show large variability in frontostriatal cognitive performance. Such variability was not associated with $D_1$ receptors in any region. This contrasts with several PET studies reporting associations between executive processes and $D_1$ receptor density in prefrontal cortex and striatum in disorders associated with dopamine dysfunction, such as schizophrenia and Huntington’s disease (Abi-Dargham et al 2002; Lawrence et al 1998; Okubo et al 1997). These findings also contrasts with studies in rodents showing involvement of $D_1$ receptors in behavioral set-shifting (Floresco and Magyar 2006; Ragozzino 2002).
Although D₁ receptors are proposed to play a critical role in cognitive stability of prefrontal neural networks (Bilder et al 2004; Durstewitz et al 2000), as required in maintenance tasks (e.g. delayed-response tasks), sustained attention or for representing on-going responses, they may not be critical for “plasticity” or cognitive flexibility (but note studies above in rodents), processes which were largely required in the current study. Recently, D₂ receptors in cortex (anterior cingulate) were associated with error parameters on the Wisconsin task in healthy subjects (Lumme et al 2007). Whether D₂ receptors, which may be more important for “resetting/updating”, flexibility or behavioral switching functions (see Bilder et al 2004; Cools 2006; Seamans and Yang 2004) are associated with planning and set-shifting performance in PD patients remains to be seen.

In contrast, impairment in a measure of executive function (Wisconsin categories achieved) in PD patients was associated with pre-synaptic dopamine loss in the putamen but not the caudate nucleus, a relationship not due to age or disease severity. This relationship was observed even after correction of the false discovery rate (Benjamini and Hochberg 1995), which is notable as many previous studies reporting similar relationships have not adequately controlled for multiple comparisons. Such an association between putamen Ki and Wisconsin performance is consistent with several PET and SPECT studies showing associations between striatal (particularly caudate nucleus but also putamen) dopamine loss and memory, attention and executive impairment in PD (Bruck et al 2001; Duchesne et al 2002; Holthoff-Detto et al 1997; Marie et al 1999; Muller et al 2000; Rinne et al 2000; van Beilen et al 2008), suggesting that striatal dopamine depletion in PD may contribute to frontostriatal cognitive impairment. Further, increased dopamine release in the striatum was observed during performance on a modified card sorting task (Monchi et al 2006a). Given the assertion that a “cognitive” loop connects areas of the dorsal prefrontal cortex to the dorsal striatum (including the dorsal caudate and dorsolateral putamen) (Alexander et al 1990; Alexander et al 1986), it is surprising that dopamine synthesis in the caudate nucleus was not associated with Wisconsin performance. While the observed relationship with the putamen fits with the proposed dorsal cognitive loop, the findings are contrary to several animal and human studies reporting an association between caudate nucleus dopamine function and set-shifting ability (Marie et al 1999; Roberts et al 1994). Nevertheless, the Wisconsin test reflects executive processes other than attentional set-
shifting. A functional imaging study suggests that the putamen may play a critical role in the execution stage of a set-shift (Monchi et al 2006b). While it is possible that dopamine synthesis in the putamen may also be associated specifically with the execution stage of the card sorting task, our study does not allow us to delineate specific components of the Wisconsin test that may be modulated by putaminal dopamine synthesis.

6.5 CONCLUSION

In summary, our sample of non-demented, moderately severe PD patients showed no alteration of D₁ receptors in striatal and cortical regions. Although D₁ receptors were not associated with frontostriatal cognitive performance, dopamine loss in the putamen predicted impairment on an executive task in PD. Because of unreasonably higher $K_i$ values in white than adjacent gray matter, we strongly question whether $[^{18}\text{F}]{\text{FDOPA}}$ uptake in cortex reflects dopamine synthesis and can be meaningfully assessed in these areas.
Chapter Seven

7 General discussion and conclusions

7.1 OVERVIEW AND SUMMARY OF FINDINGS

This thesis presented two experimental PET studies broadly designed to image and quantify components of the dopamine system outside the striatum. Pre-, post- and intra-synaptic components were examined in healthy humans or those with PD, a neurological disorder characterized by dopamine dysfunction. Three radioligands were used for these purposes: \(^{18}\text{F}\)fallypride (for post-synaptic D\(_2\)-like receptors), \(^{11}\text{C}\)NNC 112 (for post-synaptic D\(_1\)-like receptors) and \(^{18}\text{F}\)FDOPA (for pre-synaptic dopamine synthesis). Furthermore, in addition to the investigation of these extrastriatal radioligands, this thesis also explored the utility of molecular imaging for examining the neurochemical basis of higher cognitive functions. It should be emphasized however, that a major goal of this thesis was to understand the complexity of PET molecular imaging through quantification and evaluation of radioligand markers of the dopamine system, rather than towards advancing an understanding of a particular clinical disorder or physiological, biological or behavioural process. Therefore, this thesis consisted of two investigative PET studies aimed at testing radioligand feasibility and implementation in human subjects.

The first PET study of this thesis applied a novel paradigm for estimating regional dopamine levels based upon the pharmacological principles of the “occupancy model”. The study had several aims: 1) to test whether the high-affinity D\(_2\) antagonist radioligand, \(^{18}\text{F}\)fallypride, was useful for estimating two modes of dopamine transmission - phasic and tonic dopamine release, initially described by Grace (1991); 2) to examine the relationship between phasic and tonic modes of dopamine transmission and their relationship with cognitive function; and 3) to examine the reproducibility of \(^{18}\text{F}\)fallypride binding in striatal and extrastriatal regions. The findings of Study 1 replicated the findings of Riccardi et al (2006) in which amphetamine-induced phasic release displaced (i.e. decreased) \(^{18}\text{F}\)fallypride binding in
the striatum as well as several extrastriatal regions, but did not replicate the findings of Riccardi et al (2008) in which AMPT-induced dopamine depletion increased regional \[^{18}F\]fallypride binding to provide an estimate of tonic or baseline levels of dopamine. No correlation was found between phasic and tonic dopamine release and only two correlations were found between regional phasic dopamine release and a measure of cognitive function – verbal fluency. Further, the findings confirmed that \[^{18}F\]fallypride has excellent reproducibility, even in extrastriatal regions, indicating that the amphetamine-induced displacement of \[^{18}F\]fallypride was not a function of test-retest variability. These findings suggest that the vulnerability of \[^{18}F\]fallypride to competition with endogenous dopamine is different depending on the mode of dopamine transmission, i.e. whether endogenous dopamine is pharmacologically increased or decreased. Although \[^{18}F\]fallypride appears to be vulnerable to amphetamine-induced increases of dopamine, the findings suggest that it may not be vulnerable (at least reliably) to endogenous dopamine depletion. Such findings have important implications for the appropriateness of \[^{18}F\]fallypride for measuring regional phasic and tonic levels of dopamine and to its extension into the study of regional dopamine concentration in neuropsychiatric and neurological disorders. Study 1 of this thesis is published in the peer-reviewed journal, Synapse (Cropley et al 2008).

Study 2, in contrast, examined the effect of a chronic, underlying deficit of dopamine (represented by PD subjects) to changes in pre- and post-synaptic components of the dopamine system in both striatal and extrastriatal (i.e. frontostriatal) regions. In this study, pre-synaptic dopamine synthesis was assessed with the commonly used radioligand, \[^{18}F\]FDOPA, while post-synaptic D\(_1\), rather than D\(_2\), receptors, were measured with the high-affinity antagonist, \[^{11}C\]NNC 112, in non-demented, moderate stage PD patients and mean age-matched healthy controls. As mentioned previously, D\(_1\), rather than D\(_2\), receptors were examined given the preferential role of cortical D\(_1\) receptors in certain executive processes. The aims of this experimental study were to investigate pre- and post-synaptic dopamine in PD within the frontostriatal circuitry and its relationship with frontostriatal cognitive function. The findings of this study showed that while D\(_1\) receptors were able to be visualised and quantified in cortex using \[^{11}C\]NNC 112, there were no regional differences in D\(_1\) receptor density between PD patients and controls on a region of interest and exploratory voxel-wise basis. This lack of D\(_1\) receptor alteration in PD, both in the striatum and extrastriatal regions, was found
despite significant striatal dopamine loss (by about 70% in the putamen) in patients, which is a cardinal feature of this neurodegenerative disease. Although PD patients did not show any overall impairment in frontostriatal cognition, there was considerable inter-subject variability in cognitive performance. Despite this variability, D₁ receptors were not associated with neuropsychological performance in any region (including on a voxel-wise basis) in both patients and controls. This may suggest that D₁ receptors are not altered to an extent to cause cognitive impairment in PD, or further, that D₁ receptors are not critically associated with frontostriatal/executive processes in PD or normal aging. As discussed in Chapter 6 of this thesis however, medication, disease status, task components and radioligand selectivity may limit these conclusions. In contrast, pre-synaptic dopamine loss in the putamen (indicated by decreased [¹⁸F]FDOPA uptake) was associated with impaired set-shifting in PD patients only, which is consistent with several PET and SPECT studies reporting associations between striatal dopamine loss and cognitive impairment in this disease (Bruck et al 2001; Duchesne et al 2002; Holthoff-Detto et al 1997; Marie et al 1999; Muller et al 2000; Rinne et al 2000; van Beilen et al 2008). The unexpected finding of higher [¹⁸F]FDOPA $K_i$ in white compared to cortical gray matter, a quantification error likely to be caused by a model violation, was an unfortunate “finding” of Study 2, as it rendered cortical [¹⁸F]FDOPA uptake uninterpretable and consequently prevented cortical dopamine synthesis and its relationship with D₁ receptors and frontostriatal cognition to be fully explored. Nevertheless, although these “extrastriatal” objectives of the study were unable to be adequately assessed, this finding relating to cortical [¹⁸F]FDOPA serves an important function as it alerts the PET and SPECT community to the deficiencies in the cortical [¹⁸F]FDOPA signal and highlights the necessity for thorough quantitation and analysis of PET and SPECT radioligands. The findings presented in Study 2 have been accepted for publication in the peer-reviewed journal, Psychiatry Research: Neuroimaging (see Appendix 3 for reprint of proof).

In summary, the results of this thesis demonstrates the potential for two relatively new radioligands targeting dopamine D₂ and D₁ post-synaptic receptors to assess the dopamine system not only within the striatum but also in extrastriatal regions. Specifically, the results of these PET studies demonstrate that [¹⁸F]fallypride can be used to examine regional D₂ receptor density but can also be used to indirectly measure phasic changes in dopamine subcortically and cortically, whereas [¹¹C]NNC 112 is
useful for D₁ receptor quantification also in the striatum and cortex, including prefrontal regions. Further, initial implementation of [¹¹C]NNC 112 in PD, which had not been previously assessed, indicated no regional alteration of D₁ receptors. Lastly, results of this thesis demonstrate the weakness of some radioligands, specifically cortical measurement of [¹⁸F]FDOPA and emphasises the pitfalls and complexity of radioligand measurement with PET and SPECT.

7.2 GENERAL DISCUSSION AND IMPLICATIONS

7.2.1 Striatal and extrastriatal assessment of dopamine function with PET

General feasibility

Until recently, most studies investigating radioligand binding of dopamine targets using molecular imaging technology have been confined to the striatum, since the striatum is a relatively large region with dense dopaminergic innervation and further, because the signal-to-noise ratios of many dopaminergic radioligands have been inadequate to measure low density dopamine targets. However, given the involvement of both mesocortical and mesolimbic dopamine to various neuropsychiatric disorders and cognitive (particularly executive) processes, there has been a growing need for suitable extrastriatal dopamine radioligands. As outlined in Chapter 1 of this thesis, several promising radioligands have been developed over the past decade that can image dopamine targets in extrastriatal areas. Therefore, a primary aim of this thesis was to image and assess dopamine function “extrastriatally” using these “newer” radioligands or using “old” radioligands in combination with higher injected activity (i.e. dose) and novel imaging analysis approaches (i.e. voxel based approaches).

In addition to the general criteria important in the development of suitable PET radiopharmaceuticals for in vivo human imaging, there are further considerations and challenges for a radioligand to be adequate for measurement of extrastriatal receptor binding or neuronal uptake (please refer to Chapter 4 for review). For instance, the binding affinity (dissociation equilibrium constant) of a radioligand must be relatively high, and much higher than the affinity of radioligands for measurement of binding in the striatum only, since extrastriatal binding sites (e.g. dopamine receptors) are only 1–10% of that found in the striatum (Hall et al 1994; Kessler et al 1993; Lidow et al
1989b). As the optimum affinity of a radioligand is closely related to the concentration of binding sites ($B_{\text{max}}$) (Halldin et al 2001), a successful radioligand for measurement in low density regions should ideally have an affinity in the subnanomolar range. Another important determinant of a suitable extrastriatal radioligand is one with a much higher ratio of specific (i.e. target) to non-specific (i.e. non-target) binding (also referred to as signal-to-noise), again due to the low concentration of receptors in these regions. A further difficulty with extrastriatal measurement with PET in general relates to the small size of most extrastriatal regions which may consequently lead to partial volume effects.

The pharmacological properties of both $[^{18}\text{F}]$fallypride and $[^{11}\text{C}]$NNC 112 and preclinical in vivo imaging studies in nonhuman primates and humans demonstrate these “extrastriatal” requirements outlined above. Both radioligands have a high affinity for their respective binding sites and show high specific to nonspecific ratios. $[^{18}\text{F}]$fallypride displays a high affinity for the $D_2/D_3$ receptor ($K_D = 30 \, \text{pM}$) (Mukherjee et al 1995), which is substantially higher than the moderate affinity displayed by the commonly used $D_2$ radioligand $[^{11}\text{C}]$raclopride ($K_D = 1000 \text{ –} 2000 \, \text{pM}$) (Seeman et al 1989). Likewise, the affinity of $[^{11}\text{C}]$NNC 112 for the $D_1$ receptor is high ($K_D = 0.18 \, \text{nM}$) (Andersen et al 1992) and is comparable to that of the $D_1$ radioligand $[^{11}\text{C}]$SCH 23390, which has reported affinities of 0.14 nM (Andersen 1988; Andersen et al 1992; Andersen et al 1985) or ~0.4 nM (Bogeso et al 1995; Chipkin et al 1988; Lawler et al 1999).

With respect to signal-to-noise, both $[^{18}\text{F}]$fallypride and $[^{11}\text{C}]$NNC 112 exhibit superior ratios than their older radioligand counterparts. $[^{18}\text{F}]$fallypride has reported target region to cerebellum (reference) radioactivity ratios of about 30 for the putamen, and between 1.8 and 6 in extrastriatal regions such as the frontal and temporal cortex, amygdala and thalamus in human brain (Mukherjee et al 2002). In comparison, region to cerebellum ratios of $[^{11}\text{C}]$raclopride are only 4–5 in the putamen (Farde et al 1987a; Wang et al 1993), 2 in the thalamus and 1.5 in frontal and temporal cortex (Wang et al 1993). $[^{18}\text{F}]$Fallypride radioactivity ratios in the present thesis are also higher than those reported with $[^{11}\text{C}]$raclopride, with ratios of approximately 26 in the putamen, 3 in the thalamus and 1.8 in the temporal cortex, 3 h after $[^{18}\text{F}]$fallypride acquisition (data not shown). Further, from visual inspection of a parametric image of $[^{18}\text{F}]$fallypride in PET Study 1 (see Chapter 5, Figure 5-1), where each pixel represents $BP_{\text{ND}}$ and not
radioactivity, D2 receptors are clearly identified in striatum as well as extrastriatal regions such as thalamus and temporal cortex. [18F]fallypride is also advantageous over the very high-affinity D2 radioligand [11C]FLB 457 (Ki = 20 pM) (Halldin et al 1995), since the washout of [18F]fallypride from the striatum is sufficiently fast enough, along with the longer half-life of [18F] (110 min), to allow quantification of receptor concentration in both striatal and extrastriatal regions. Because of the longer time for [11C]FLB 457 to equilibrate in high receptor density regions such as the striatum (Okubo et al 1999), it is only suitable for evaluation of extrastriatal receptors. Thus, the characteristics of [18F]fallypride make it a suitable candidate for regional assessment and comparison of human D2 receptors and, on the basis of the competition or ‘occupancy’ paradigm outlined in Chapter 1 and investigated in Chapter 5 of this thesis, for regional evaluation of ‘phasic’ (but possibly not tonic) levels of dopamine (see results of Chapter 5).

[11C]NNC 112 likewise shows superior signal-to-noise ratios than those obtained for another D1 radioligand, [11C]SCH 23390. With [11C]NNC 112, radioactivity ratios of human striatum, frontal cortex and nucleus accumbens to the cerebellum have reported to be approximately 4, 2 and 3, respectively (Halldin et al 1998), although Abi-Dargham et al (2000a) obtained slightly lower ROI/cerebellar activity ratios, with ratios reaching 3.7 in the putamen, 1.7–1.8 in the temporal cortex, amygdala and anterior cingulate, and 1.5 in the dorsal-lateral prefrontal cortex. The striatal to cerebellum ratio for [11C]SCH 23390 is lower than with [11C]NNC 112, with a value of approximately 3 (Farde et al 1987a). In the present thesis, region-to-cerebellum [11C]NNC 112 radioactivity ratios in elderly healthy subjects were roughly 3.5 in the putamen and 1.5 in frontal and parietal cortex (data not shown in Chapter 6). Nevertheless, although the parametric image of [11C]NNC 112 BP_{ND} shows D1 receptor binding in the striatum and in extrastriatal regions such as the thalamus, temporal and frontal cortex (see Chapter 6, Figure 6-2), the returned extrastriatal BP_{ND} values are still close to zero (see Chapter 6, Table 6-4), indicating that D1 radioligands with higher signal-to-noise ratios are still needed. Although human studies of [11C]NNC 112 are limited, and only two other clinical investigations of [11C]NNC 112 have been reported (Abi-Dargham et al 2002; Narendran et al 2005a), initial PET studies of [11C]NNC 112 have indicated its feasibility for assessment of extrastriatal D1 receptors in health and disease. The present thesis used [11C]NNC 112 for this purpose, to measure striatal and cortical D1 receptors.
in a clinical population – PD. However, recent studies in monkeys and humans have demonstrated that the in vivo selectivity of $[^{11}\text{C}]$NNC 112 in cortex is poorer than previously thought (Ekelund et al 2007; Slifstein et al 2007) (see following sections for further discussion) – a characteristic not known at the outset of this thesis, and, therefore, will require cautious interpretation of cortical data using $[^{11}\text{C}]$NNC 112 in clinical populations, including PD.

### 7.2.2 Discussion of “positive” striatal and extrastriatal imaging findings

As observed in Study 1 of this thesis, $[^{18}\text{F}]$fallypride binding to D$_2$-like receptors were reduced following oral amphetamine in both striatal and most extrastriatal regions in healthy volunteers. This amphetamine-induced displacement of D$_2$ radioligand binding in healthy humans is consistent with a previous report using $[^{18}\text{F}]$fallypride and oral amphetamine (Riccardi et al 2006), as well as studies using other striatal benzamide radioligands, ($[^{11}\text{C}]$raclopride or $[^{123}\text{I}]$IBZM), with either intravenous (Drevets et al 2001; Kegeles et al 1999; Laruelle et al 1995; Martinez et al 2003) or oral (Boileau et al 2006; Boileau et al 2007; Cardenas et al 2004; Leyton et al 2002) doses. Other psychostimulant challenges such as methylphenidate, have also reduced D$_2$ radioligand receptor availability in healthy human striatum (Booij et al 1997; Volkow et al 1994b; Wang et al 1999) as well as extrastriatal regions (Montgomery et al 2007). We observed relatively high displacement of $[^{18}\text{F}]$fallypride binding in striatal subdivisions as well as the substantia nigra and orbitofrontal cortex (see Chapter 5), which suggests that $[^{18}\text{F}]$fallypride is advantageous over other radioligands for measurement of regional psychostimulant-induced change in binding, as other tracers allow only striatal (i.e. $[^{11}\text{C}]$raclopride, $[^{123}\text{I}]$IBZM) or extrastriatal (i.e. $[^{11}\text{C}]$FLB 457) measurements to be made.

Amphetamine-induced displacement of $[^{18}\text{F}]$fallypride binding is consistent with the “occupancy model” (see Chapter 1), which is based on the principle of competition between endogenous dopamine and radioligand for binding to D$_2$ receptors (Laruelle 2000). On this basis it is proposed that our findings of decreased $[^{18}\text{F}]$fallypride binding relative to the baseline scan is due to the increased competition between $[^{18}\text{F}]$fallypride and the amphetamine-induced increases in extracellular dopamine for D$_2$ receptors,
therefore providing an estimate of intrasynaptic dopamine concentration or stimulant-induced dopamine release. Grace (1991), described a mode of dopamine transmission – defined as phasic dopamine release, as a transient and high-amplitude release of dopamine produced by burst firing of dopamine neurons. Although the strict electrophysiological criteria for phasic dopamine release assigned by Grace (1991) may be different to stimulant-induced dopamine release, radioligand displacement studies probably loosely represent this phasic activity mode (Grace et al 2007). As such, amphetamine-induced \[^{18}\text{F}]\text{fallypride} displacement has been loosely described as “phasic” dopamine release in this thesis. More accurately however, this imaging measurement reflects the net effect of dopamine release and reuptake – as well as the amount of transmitter that diffuses to the D\(_2\) receptor, whether located within or adjacent to the synapse.

Although reduction of radioligand binding following amphetamine challenge is generally ascribed to competitive interactions between the dopamine agonist and ligand, several data question this simple occupancy model to explain such changes and point to non-competitive interactions with endogenous dopamine (see Chapter 1). As discussed in Chapter 1, agonist-mediated internalization of D\(_2\) receptors is an alternative model to account for the change in radioligand binding observed following dopamine manipulations. According to this model, released dopamine (from agents such as amphetamine) shifts D\(_2\) receptors from the externalized cell membrane to the internalized endosomal compartment, and certain radioligands (i.e. those with low lipophilicity such as benzamides) lose access to the internalized receptors which translates into reduced radioligand binding (Laruelle 2000; Sun et al 2003). Although such a mechanism has been proposed to account for the reduced \[^{3}\text{H}]\text{raclopride} binding in rat striatum after amphetamine challenge, where it was demonstrated that the amphetamine-induced decreases in \[^{3}\text{H}]\text{raclopride} binding were caused by a reduction in D\(_2\) receptor density (\(B_{\text{max}}\)) with no change in affinity (\(K_d\)) (Sun et al 2003), the mechanism is less clear \textit{in vivo}, such that methamphetamine or amphetamine-induced reduction in \[^{11}\text{C}]\text{raclopride} binding has been reported to be due solely to an increase (>100\%) in \[^{11}\text{C}]\text{raclopride} \(K_D\) (reflecting solely competitive mechanisms) (Doudet and Holden 2003) and to both a decrease (28\%) and increase (35\%) in \(B_{\text{max}}\) and \(K_D\), respectively (Ginovart et al 2004). Further, agonist-mediated internalization and subsequent endosomal trapping may differentially affect radioligand binding, depending
on the ligand properties (Laruelle 2000). The ability of $[^{18}\text{F}]$fallypride to diffuse within the cell membrane and bind to internalized receptors is unknown. As such, because data supporting the agonist-mediated internalization model are, at present, scarce, and because available data examine the effect of $D_2$ internalization on raclopride (or spiperone, see Chugani et al 1988) binding and not fallypride binding, our observation of amphetamine-induced displacement of $[^{18}\text{F}]$fallypride binding is interpreted within the framework of the occupancy model, and not the internalization model. Nevertheless, further evaluation of the effects of agonist-mediated $D_2$ receptor trafficking (including not only internalization but G-protein uncoupling and dimerization) on in vivo radioligand binding is warranted.

7.2.3 Discussion of “negative” striatal and extrastriatal imaging findings

Lack of effect of AMPT on regional $[^{18}\text{F}]$fallypride binding

A major finding of PET Study 1 was that pharmacological depletion of dopamine with AMPT failed to modulate $[^{18}\text{F}]$fallypride binding in healthy humans (see Chapter 5). This occurred despite the sensitivity of $[^{18}\text{F}]$fallypride to acute pharmacological release of synaptic dopamine with amphetamine in the same subjects. Furthermore, this lack of AMPT-induced change in $D_2$ receptor availability using $[^{18}\text{F}]$fallypride is in contrast with several previous PET and SPECT studies using AMPT and $D_2$ receptor radioligands, including $[^{18}\text{F}]$fallypride (Fujita et al 2000; Laruelle et al 1997a; Riccardi et al 2008; Verhoeff et al 2003; Verhoeff et al 2002; Verhoeff et al 2001). In light of these “negative” findings and of the apparent contrasting sensitivity of $[^{18}\text{F}]$fallypride to changes in dopamine, one must consider whether such null findings represent an actual “true” effect (i.e. $[^{18}\text{F}]$fallypride is not vulnerable to endogenous dopamine depletion) or rather are the result of methodological issues or alternative pharmacological mechanisms. Several possible reasons for the discrepant AMPT findings of PET Study 1 have been discussed within Chapter 5 of this thesis. As recognized in that chapter, many of these reasons are speculative, providing no definitive explanation of the findings, but are intended to raise awareness of the multitude of factors that contribute to the interpretation of receptor imaging data.

For instance, as was discussed within Chapter 5 of this thesis, the lack of AMPT-induced change in $[^{18}\text{F}]$fallypride binding observed in PET Study 1 was probably not
related to the dose of AMPT used (3g/70kg/day for 44 h). This dose was similar to that used in other studies and resulted in comparable steady-state levels of AMPT in plasma to those obtained previously (Fujita et al 2000; Laruelle et al 1997a; Verhoeff et al 2001). Given these equivalencies in dose and plasma levels to previous studies that observed significant increases in D₂ receptor radioligand binding, it seems reasonable to assume that dopamine levels were depleted to a sufficient extent in PET Study 1, and that the null findings were not due to insufficient dopamine depletion. Nevertheless, the possibility that there was insufficient central dopamine depletion in Study 1, at least to an extent to modulate \[^{18}F\]fallypride binding to D₂ receptors, cannot be ruled out. For example, it may be possible that dopamine is less potent at competing with \[^{18}F\]fallypride for D₂ receptor binding and as a consequence, requires greater endogenous depletion of dopamine in order to modulate its binding in comparison to lower-affinity D₂ radioligands such as \[^{11}C\]raclopride and \[^{123}I\]IBZM. As dopamine is more potent at competing with \[^{3}H\]raclopride than \[^{3}H\]spiperone binding to D₂ receptors (Hall et al 1990), it may be possible that this is also the case with \[^{18}F\]fallypride. Another consideration related to the notion of insufficient dopamine depletion is that of AMPT related side-effects. The healthy subjects in Study 1 experienced relatively weak AMPT effects, as indicated subjectively (as assessed by AMPT-induced mood changes), behaviourally (as assessed by AMPT-induced cognitive changes) and objectively (as assessed by the number of withdrawals or necessity for treatment to complete the scan), which may suggest insufficient central dopamine depletion in these subjects. However, characteristics of the study sample and/or variability in the scanning interval may have also contributed to the apparent weaker effects, rather than insufficient or non-optimal dopamine depletion.

As discussed in Chapter 5, one notable source of difference between Study 1 and previous molecular imaging studies with AMPT (e.g. Fujita et al 2000; Laruelle et al 1997a; Riccardi et al 2008; Verhoeff et al 2001) was that the current study administered a single dose of amphetamine prior to AMPT administration. This methodological difference occurred because an objective of Study 1 was to examine, in the same subjects, both amphetamine-induced and AMPT-induced changes in dopamine release. Although the interval between the two exposures (amphetamine and AMPT) far exceeded the half-life and subsequent clearance of amphetamine, and the occurrence of a sensitization-like effect on dopamine transmission (see Boileau et al 2006) would have
been unlikely, prior amphetamine exposure should nevertheless be regarded as a caveat of Study 1. A further methodological consideration and difference between the studies concerns the time interval between baseline and challenge studies. Previous studies have conducted baseline and AMPT scans within a very short time interval, generally one to two days after their baseline scan (Fujita et al 2000; Laruelle et al 1997a; Riccardi et al 2008; Verhoeff et al 2001). Due to the four-scan protocol of the current study (test-retest, amphetamine and AMPT), and to scheduling restrictions and radio-synthesis difficulties, the interval between baseline and AMPT scans varied amongst subjects, approximately ranging between 6 weeks and 9 months. While a relatively short test-retest interval showed little variability in baseline \([^{18}\text{F}]\)fallypride binding, it is unknown whether a longer interval between \([^{18}\text{F}]\)fallypride scans would be a confound. It is possible however, that such variability in the interval between baseline and AMPT neurocognitive assessment may have contributed to the variable AMPT-induced cognitive effects, and decreased the likelihood of detecting overall cognitive impairment. Nevertheless, it should be noted that a variable interval between baseline and amphetamine studies (approximate range was 1 week to 10 months) did not affect the ability for acute amphetamine administration to significantly and reliably displace \([^{18}\text{F}]\)fallypride binding. Therefore, the impact of scan interval on AMPT-induced \([^{18}\text{F}]\)fallypride binding in this study is uncertain, although interval length should be considered in future studies.

Of note in Study 1 was the inter-subject variability in AMPT-induced \([^{18}\text{F}]\)fallypride \(BP_{ND}\), although it should be noted that there was a trend for decreased specific binding or no change at all, rather than the expected increase. The cause of the paradoxical decreases in binding potential is unclear, particularly in light of a recent study reporting increased AMPT-induced binding with \([^{18}\text{F}]\)fallypride in some (but not all) regions (Riccardi et al 2008). Careful re-examination of the imaging data and analysis procedures verified that the paradoxical decreases were not due to human error or inaccuracies in analysis, at least within our ability to detect so. The imaging data underwent careful motion correction/re-alignment and furthermore, the same analysis procedures were carried out for all PET studies (i.e. baseline, amphetamine and AMPT scans). Therefore, we are confident that the current findings are not a result of inaccuracies in PET analysis. Rather, of greater bearing to the variable or paradoxical findings may be the characteristics of the study sample, radioligand characteristics, or
alternative mechanisms influencing receptor-ligand interactions. The sample of Study 1 was similar to the samples of previous studies, at least on the basis of the provided information. However, it is unknown to what degree, if any, other characteristics such as gender, race, or tyrosine hydroxylase genes may have contributed to the variable $[^{18}\text{F}]$fallypride $BP_{ND}$ with AMPT. For instance, there may be inter-individual differences in the pharmacodynamic effects of AMPT on tyrosine hydroxylase, or in endogenous baseline dopamine levels, all of which may have affected the potency of competition for D$_2$ receptor occupation and the in vivo modulation of change in $[^{18}\text{F}]$fallypride binding, as well as affect the pharmacodynamic response to AMPT. At present, a functional polymorphism in the tyrosine hydroxylase gene (the TCAT$_n$ tetranucleotide repeat polymorphism), consisting of at least five alleles (Polymeropoulos et al 1991) has been discovered and has been associated with catecholamine turnover, specifically in relation to the A1 allele and upregulation of dopamine turnover (Wei et al 1997). Although unknown at present, such allelic variants of the tyrosine hydroxylase gene may prove useful in predicting individual responses to dopamine manipulation with AMPT as our knowledge of this, and other, functional polymorphisms increases.

The findings of Study 1, that sustained dopamine depletion has no effect, or a trend for a paradoxical effect on $[^{18}\text{F}]$fallypride binding, is inconsistent within the framework of the “occupancy model”, which predicts that changes in specific binding of a dopamine D$_2$ radioligand reflect changes in endogenous dopamine (Laruelle 2000). Consideration of other factors that might be involved in the modulation of receptor availability is therefore necessary. There are a number of “unknowns” that could potentially influence the in vivo sensitivity of a radioligand to changes in dopamine, such as the proportion of receptors in the high and low affinity state, the proportion of synaptic versus extrasynaptic and internalized and non-internalized receptors, the proportion of D$_2$ receptors existing in the monomeric or dimeric configuration and differences in radioligand binding sites. Clearly, further work is required to elucidate potential relationships between such complex factors and vulnerability to endogenous dopamine competition, particularly dopamine depletion. It should be noted that the high affinity of $[^{18}\text{F}]$fallypride for D$_2$ receptors would not have played a role in the resistance displayed by it to endogenous dopamine depletion. This is because the affinity of a radioligand will not affect its vulnerability to competition by endogenous dopamine so long as the
radioligand is given at tracer dose and equilibrium conditions are met, i.e. dopamine levels are constant during the time frame of the PET study (Laruelle 2000). In AMPT studies tracer doses were given and equilibrium conditions would have been achieved with the dopamine depletion paradigm. However, in the case of stimulant-induced dopamine release with amphetamine, dopamine changes dynamically over time and the equilibrium condition is not achieved. In these instances, radioligand affinity may influence the sensitivity of a radioligand to displacement by a dopamine pulse, with low affinity radioligands being more vulnerable to dopamine competition (Endres and Carson 1998). Thus, it would have been more likely that \([^{18}F]{\text{fallypride}}\), with its high affinity for \(D_2\) receptors, to not be displaced by a transient amphetamine-induced increase in dopamine levels but to show increases in binding after a sustained decrease in dopamine, as has previously been demonstrated with the high-affinity radioligand \([^{123}I]{\text{epidepride}}\) (al-Tikriti et al 1994; Fujita et al 2000).

In summary, the lack of effect of AMPT-induced dopamine depletion on regional \([^{18}F]{\text{fallypride}}\) binding is intriguing. While this lack of effect is at odds with previous AMPT imaging studies, and is inconsistent with changes predicted by the occupancy model, there are no reasonable methodological or experimental explanations for our current findings. Clearly, further work is required to re-assess whether \([^{18}F]{\text{fallypride}}\) is in fact vulnerable to dopamine depletion and to explore other pharmacological or physiological factors that might be involved in the modulation of receptor availability following dopamine depletion.

Lack of regional \(D_1\) receptor alteration using \([^{11}C]{\text{NNC 112}}\) in PD

As reported in Chapter 6 of this thesis, Study 2 did not detect any differences in \([^{11}C]{\text{NNC 112}}\) binding in PD patients compared to age-matched healthy elderly controls. This “negative” finding was observed throughout the entire brain, including frontostriatal regions, despite PD patients evidencing significantly reduced dopamine metabolism in the striatum. A logical interpretation of this finding is that PD without dementia does not alter the density of regional \(D_1\) receptors. A tentative extension of this interpretation is that progressive degeneration of dopamine neurons (nigrostriatal and possibly mesocortical and mesolimbic), resulting in sustained and marked dopamine depletion in the striatum and, perhaps, cortical areas (see Chapter 1) does not result in altered (either increased or decreased) \(D_1\) receptors. However, again, as with all
“negative” studies, methodological issues or alternative interpretations should be considered within the context of these findings.

Results of Study 2 show that not only were there no significant differences in $^{11}$C]NNC 112 binding between PD and controls, there were also no meaningful trends for any differences in binding values, indicating that even with a larger sample the same result would likely eventuate. Examination of $^{11}$C]NNC 112 $BP_{ND}$ from a region of interest analysis show that values are very similar between patients and controls and considerably far from significant, with $p$-values ranging from 0.26 – 0.76. Furthermore, exploratory voxel-wise analysis with statistical parametric mapping showed no significantly different (FDR corrected) voxels anywhere in the brain. Therefore, in our sample of moderately severe, non-demented PD patients, $^{11}$C]NNC 112 binding to dopamine $D_1$ receptors were not, and showed no sign of, being altered. This finding of lack of $D_1$ receptor alteration is in agreement with several other $in vivo$ and $in vitro$ $D_1$ receptor binding studies in PD. Using the $D_1$ radioligand $^{11}$C]SCH 23390, $D_1$ receptor density in the striatum and orbitofrontal cortex was reported unchanged in early (Hoehn and Yahr stage I and II) (Ouchi et al 1999a) and early to moderate (Hoehn and Yahr I – IV) (Shinotoh et al 1993) PD. Likewise, several $in vitro$ studies have reported no change of $D_1$ receptors in the putamen of PD (Cash et al 1987; Cortes et al 1989; Pierot et al 1988; Pimoule et al 1985; Raisman et al 1985), although $D_1$ receptor upregulation has also been reported (Hurley et al 2001; Raisman et al 1985; Rinne et al 1985; Seeman et al 1987b). In extrastriatal regions such as the substantia nigra, $D_1$ receptors have been reported to be unchanged (Cash et al 1987; Cortes et al 1989; Hurley et al 2001) but also decreased (Cash et al 1987; Rinne et al 1985). Therefore, at least $in vivo$, the existing literature indicates that striatal and extrastriatal $D_1$ receptors are not altered in non-demented PD.

Whether the findings of Study 2 indicate that sustained dopamine depletion does not alter $D_1$ receptors (as in denervation supersensitivity) is unclear. In our sample of PD patients, $D_1$ receptors were unchanged despite evidence of significantly reduced dopamine metabolism (indexed by $[^{18}F]$FDOPA uptake) in the striatum. In addition to this, they showed no relationship between striatal pre-synaptic dopamine synthesis and post-synaptic $D_1$ receptors, such that dopamine loss was not associated with a relative elevation or reduction of $D_1$ receptors. In the cortex, such a relationship was unable to
be assessed in the current thesis due to inadequacies in cortical $[^{18}F]$FDOPA measurement. This is inconsistent with Ouchi et al (1999a), who reported an inverse correlation between pre-synaptic dopamine transporter binding and post-synaptic D$_1$ receptors in early PD. However, several animal studies have not demonstrated D$_1$ receptor supersensitivity from basal ganglia dopamine depletion or lesion of dopaminergic nigrostriatal neurons (Graham et al 1990a; Graham et al 1990b; Trugman et al 1990). While early, de novo PD may be characterized by relative upregulation of post-synaptic dopamine D$_1$ receptors in compensation for pre-synaptic neuronal degeneration (as implied by Ouchi et al 1999a), such an inverse relationship may not be apparent in later stages of the disease.

As mentioned in Chapter 6, due to feasibility issues PD patients remained on their normal treatment for the $[^{11}C]$NNC 112 PET scan. This consisted of L-dopa-containing medications (e.g. Sinemet® or Stalevo®) in all but one patient, amantadine, which acts by enhancing release of dopamine from pre-synaptic terminals (Rezak 2007) in one patient, and pramipexole in 47% of patients. Pramipexole is a dopamine agonist selective to the D$_2$-type receptor and has a preferential affinity for the D$_3$ subtype (Piercey 1998). No patient was taking the dopamine agonists pergolide or cabergoline, which have a relatively high affinity for D$_1$ receptors (Kvernmo et al 2006). It is acknowledged however that Study 2 is subject to criticism on possible drug interference as a confounding effect on D$_1$ receptor binding parameters. As stated in Chapter 6, the medications our PD patients were taking are not known to directly interact with D$_1$ receptors. However, as administration of L-dopa increases synaptic dopamine concentrations (Tedroff et al 1996b), it is possible that this increase in extracellular dopamine may have affected D$_1$ receptor concentration per se. Nevertheless, what this would have involved is unclear. Possibly the generation of additional dopamine may have down-regulated D$_1$ receptors in PD patients to that of control levels (i.e. normalized), so that an up-regulation of D$_1$ receptors, or relative denervation supersensitivity of D$_1$ receptors (as was observed by Ouchi et al 1999a) was masked. Such an effect is loosely supported by the observation of D$_1$ receptor down-regulation from long-term L-dopa exposure (Turjanski et al 1997). On the other hand, in post-mortem PD patients on L-dopa at or close to the time of death, D$_1$ receptor density was either increased (rather than decreased) or unchanged (Cash et al 1987; Cortes et al 1989; Hurley et al 2001; Pierot et al 1988; Pimoule et al 1985; Raisman et al 1985;
Rinne et al 1985; Seeman et al 1987b). Therefore, while medication should be considered as a caveat of Study 2, the effect, if any, of such treatment on D₁ receptor binding estimates is unclear. To date, the literature suggests that medication would have had little or no effect on D₁ receptor density, or would have resulted in increased receptor levels, which was not noted in the current thesis. As such, we propose that in early to moderate, non-demented PD, D₁ receptors are not altered, despite treatment with anti-Parkinson medication.

Another methodological issue that warrants discussion in relation to the current D₁ receptor findings concerns the selectivity profiles of the presently available PET D₁ radioligands, [¹¹C]NNC 112 and [¹¹C]SCH 23390. As mentioned in Chapter 6, a weakness recently found for both of these tracers is their relatively poor D₁ to 5-HT₂A receptor selectivity ratios. Although it was previously known that the second highest affinity of both [¹¹C]NNC 112 and [¹¹C]SCH 23390 is to the 5-HT₂A receptor, previously available data (at the outset of this thesis) suggested that the contribution of 5-HT₂A receptors to [¹¹C]NNC 112 and [¹¹C]SCH 23390 binding, even in cortex, should be negligible. For [¹¹C]NNC 112, the selectivity ratio for D₁ versus 5-HT₂A was previously reported in vitro to be 100 (Andersen et al 1992), while the selectivity ratio of [¹¹C]SCH 23390 was reported in vitro to be 250 by the same investigators (Andersen et al 1992), although a smaller ratio of 60 has also been reported (Laruelle et al 1991). Even though the density of 5-HT₂A receptors is approximately twice that of D₁ receptors in primate cortex (Lidow et al 1989a), such D₁ to 5-HT₂A selectivity ratios of about 100 would result in an insignificant contribution (~2%) of 5-HT₂A binding to the cortical signal of both [¹¹C]NNC 112 and [¹¹C]SCH 23390. Previous in vivo data also suggested negligible contribution of 5-HT₂A receptors to the cortical signal of both these radioligands (Halldin et al 1998; Suhara et al 1992). Based on this data, we assumed [¹¹C]NNC 112 to have good selectivity for D₁ receptors in cortex. Very recent studies in non-human primates and humans however, demonstrate considerably smaller selectivity ratios than 100-fold for both D₁ receptor radioligands. In baboons, about 25% of the cortical uptake of both [¹¹C]NNC 112 and [¹¹C]SCH 23390 is attributed to 5-HT₂A receptor binding (Ekelund et al 2007). This finding was confirmed in humans for [¹¹C]NNC 112, where approximately 20 – 30% of the cortical specific signal is to 5-HT₂A receptors (Slifstein et al 2007). Therefore, selectivity of both [¹¹C]NNC 112 and [¹¹C]SCH 23390 is proposed to be about 10-fold (Ekelund et al 2007; Slifstein et al
2007), as opposed to 100-fold as was previously reported. Consequently, the cortical binding of \([^{11}\text{C}]\text{NNC 112}\) in our sample of healthy subjects and PD patients in Study 2 would have been due (by about \(\frac{1}{4}\)) to binding to 5-HT\(_{2A}\) receptors. Given that no differences in regional \([^{11}\text{C}]\text{NNC 112}\) binding were observed between PD patients and controls, caution need not be exercised in the interpretation of our results. Nevertheless, it may be possible that slight differences in cortical D\(_1\) receptors were masked by binding to cortical 5-HT\(_{2A}\) receptors. These findings demonstrate how \textit{in vitro} and \textit{in vivo} situations may differ, and the need for developing more selective radioligands for the D\(_1\) receptor.

In summary, Study 2 found no alteration of D\(_1\) receptors, reflected by regional \([^{11}\text{C}]\text{NNC 112}\) binding, in the striatum and extrastriatal regions, in non-demented, moderate stage PD. Despite patients maintaining their normal anti-Parkinson treatment, and despite a drawback associated with the selectivity of \([^{11}\text{C}]\text{NNC 112}\), we are confident that this finding is not a “false negative”, and does indicate a relatively sparing of D\(_1\) receptor density in PD, at least in our sample of volunteers.

7.2.4 Quantification issues: error in the quantification of cortical \([^{18}\text{F}]\text{FDOPA}\)

Quantitative or mathematical modeling of PET and SPECT data is required in order to relate the measured radioactivity of the injected radioligand to a physiological parameter of interest, e.g. binding of the radioligand to a specific receptor, or uptake or “trapping” of the radioligand in the observed system. In this way, properties of the molecular target (e.g. receptor or enzyme) and its interaction with the radioligand is defined, without the confounding influence of factors such as blood flow, concentration of radioligand and subject weight to the obtained radioactivity. A number of quantitative kinetic models that describe the physiological behaviour of a radioligand in the system exist for this purpose (for review see Chapter 4). In these models, several assumptions are usually made in order to reduce the number of kinetic parameters to yield reliable results. As mentioned by Kegeles and Mann (1997) in their review of \textit{in vivo} receptor imaging of neurotransmitter systems, attempts at reconciling discrepant or unexpected imaging results has often lead to examinations of the assumptions underlying mathematical modeling of specific \textit{in vivo} PET and SPECT data. In this
thesis, such an examination of modeling assumptions took place, due to the unexpected finding in Study 2 of higher $[^{18}F]$FDOPA $K_i$ in white matter, compared to cortical gray matter regions. As discussed in Chapter 6, greater $K_i$ – the outcome measure representing the influx constant of $[^{18}F]$FDOPA, in white as compared to adjacent gray matter, is physiologically unreasonable since AADC, the enzyme which synthesizes $[^{18}F]$FDOPA to $[^{18}F]$Fluorodopamine, is minimally present in white matter. Therefore, this finding, observed in both patients and controls, suggested either experimenter error in the $[^{18}F]$FDOPA PET analysis or possible inability of the graphical model or $[^{18}F]$FDOPA itself to accurately measure cortical AADC activity. Methodological or experimenter error is extremely unlikely however, as standard imaging and analysis methodologies were used for $[^{18}F]$FDOPA measurement, and typical $K_i$ values were observed in the striatum of PD patients and controls. As expected, those with PD showed considerably lower $[^{18}F]$FDOPA uptake, by 70% in the putamen and 36% in the caudate nucleus, than healthy elderly controls, a finding which is well established in PD patients and consistent with the known degeneration of nigrostriatal dopamine neurons. While this finding is by no means novel, it is important as it indicates that the quantification of the $[^{18}F]$FDOPA PET signal was, in respect to experimenter computation, accurate.

The Patlak graphical analysis using a reference method (occipital cortex) in place of a plasma input curve (Patlak and Blasberg 1985) was used to estimate regional $K_i$. This graphical analysis is based on a two-compartment model for irreversible radioligands ($k_4=0$), i.e. the transfer of $[^{18}F]$FDOPA to $[^{18}F]$Fluorodopamine in target regions (e.g. striatum or frontal cortex) is assumed unidirectional, such that it is “trapped” within the system. With a reference tissue input, it is assumed that there is a region in which the radioligand only behaves in a reversible, and not irreversible, manner, i.e. $[^{18}F]$FDOPA in the occipital cortex or cerebellum is reversibly exchangeable with blood and undergoes negligible conversion to $[^{18}F]$Fluorodopamine because of minimal AADC in these regions. Another assumption of the Patlak plot using a reference method is that the ratio of activity in nondisplaceable (reversible) compartments in target compared to reference regions is constant after $t^*$ ($Me(t) \propto Mr(t)$ after $t^*$) (see Patlak and Blasberg 1985). As discussed in Chapter 6, this model assumption would have been violated, since the kinetics of $[^{18}F]$FDOPA were markedly different between white matter and the
occipital cortex (which contains both gray and white matter), thus causing discrepant kinetics between both cortical gray and white matter to that of the reference region. This violation likely caused the erroneously and paradoxically greater $K_i$ values in white matter. While this finding was unfortunate and disappointing, consequently forcing us to abandon measurement of pre-synaptic dopamine function in cortex and its association with cortical post-synaptic $D_1$ receptors and cognitive function (which was a primary objective of the current thesis), it does highlight the importance for research groups to carefully evaluate PET and SPECT data and the models (and their underlying assumptions) used for their analysis. Over recent years, several groups have reported to reliably measure $[^{18}\text{F}]$FDOPA $K_i$ in human cortex and have recommended $[^{18}\text{F}]$FDOPA as a suitable marker of cortical pre-synaptic dopamine synthesis and storage (Bruck et al 2005; Ito et al 2002; Kaasinen et al 2001; Nagano et al 2000; Rakshi et al 1999; Rinne et al 2000). Because these studies also used a Patlak reference tissue methodology, it is possible that a model violation also occurred in their data, although this is speculative as no comparisons of $[^{18}\text{F}]$FDOPA uptake between white and adjacent gray matter were provided. Nevertheless, given the widespread use of the Patlak reference method for $[^{18}\text{F}]$FDOPA quantification and the growing reports of cortical or extrastriatal $[^{18}\text{F}]$FDOPA measurement using these techniques, the scientific community should be alerted to this likely error in cortical $[^{18}\text{F}]$FDOPA quantification. In addition to this quantification error, we question the ability for $[^{18}\text{F}]$FDOPA to provide a specific PET signal due to the reasons outlined and discussed in the previous chapter. Therefore, despite being unable to address potentially important relationships between cortical pre- and post-synaptic dopamine function (such as the effect of cortical pre-synaptic dopamine denervation on post-synaptic dopamine $D_1$ receptors), the current thesis did evaluate the suitability of $[^{18}\text{F}]$FDOPA for extrastriatal measurement, of which we conclude that $[^{18}\text{F}]$FDOPA has serious shortcomings as a specific marker for cortical dopamine synthesis.

### 7.2.5 Using molecular imaging as a means to explore the neurochemical underpinnings of human cognitive function

The role of neurochemicals in higher cognitive processes is an expanding research pursuit, in part due to widespread recognition of cognitive impairment in many neurological and psychiatric disorders involving neurochemical disturbance and the
discovery of pharmacological treatments effective at sparring or ameliorating various cognitive problems. The involvement of dopamine in cognition, particularly for “prefrontal”, executive or frontostriatal processes, is to date probably the most studied and understood of all the CNS neurotransmitter systems, experimentally dating back to the pioneering study of Brozoski and colleagues (1979), in which 6-hydroxydopamine injections in monkey prefrontal cortex were found to impair working memory. Since then, subsequent studies in non-human primates and rodents using electrophysiological, neurochemical, pharmacological and behavioral methodologies (Cai and Arnsten 1997; Sawaguchi and Goldman-Rakic 1991; Sawaguchi and Goldman-Rakic 1994; Watanabe et al 1997; Young et al 1998), pharmacological challenge and functional imaging studies in humans (Harrison et al 2004; Mattay et al 2000; Mehta et al 2004; Mehta et al 2003; Mehta et al 2000; Mehta et al 1999; Mehta et al 2001; Muller et al 1998; Roesch-Ely et al 2005) as well as computational models of dopamine function (Dreher et al 2002; Durstewitz et al 1999; Durstewitz and Seamans 2002), have all demonstrated the importance of dopamine for a variety of higher cognitive processes (see Chapter 2 for brief overview). As reviewed in Chapter 2 of this thesis, a recent application of molecular imaging technology using PET and SPECT is to explore the association between neurotransmitter systems in vivo and human cognitive processes. The dopamine system has again been the most investigated, probably due to the greater number and availability of dopaminergic radioligands as well as to the strong rationale provided from previous studies for investigating such links. Chapter 2, which has in part been published in Biological Psychiatry (see Cropley et al 2006a), reviewed existing molecular imaging studies that examined the relationship between cognitive processes and pre-, intra-, and post-synaptic components of the dopamine system in human volunteers. As described in that chapter, several promising relationships between dopamine markers and human cognition have been identified, particularly in association with pre-synaptic dopaminergic markers in PD, markers of D1 and D2 receptors in healthy aging and schizophrenia, as well as with stimulant-induced or “phasic” dopamine release in healthy volunteers. Given such promising relationships, the current thesis also explored the association between human cognitive processes and phasic and tonic dopamine release (Study 1), and pre-synaptic dopamine synthesis and post-synaptic D1 receptors (Study 2).

We observed several significant relationships, all corrected for multiple cognitive
processes and brain regions, between change in specific dopamine markers and cognitive processes, giving some credence to previous imaging studies and the usefulness of this technique for examining the role of dopamine in human cognitive, particularly executive, processes. Study 1 observed a significant relationship between amphetamine-induced (phasic) dopamine release and verbal fluency in the thalamus and substantia nigra, whereas Study 2 found a significant positive relationship between pre-synaptic dopamine synthesis in the putamen of PD patients and a measure of set-shifting/executive function. Our finding linking thalamic dopamine release to verbal fluency was speculated to arise indirectly via neuronal connections between the striatum, thalamus and frontal cortex (Alexander et al 1990) and is supported indirectly through observations of thalamic activation during verbal fluency performance (Frith et al 1995) and studies showing associations between verbal fluency and striatal and frontal dopamine markers (Lawrence et al 1998; Rinne et al 2000). While no experimental studies exist to support a link between dopamine release in the substantia nigra and verbal fluency, we speculated that it may reflect a relationship with overall activity of dopamine neurons, given dopamine cell bodies originate in this brainstem region (see Chapter 5). Our finding linking loss of pre-synaptic dopamine synthesis in the putamen of PD patients to poor performance on the Wisconsin card sorting task suggests that striatal dopamine depletion in PD contributes to frontostriatal or executive impairment and is consistent with previous molecular imaging studies reporting striatal dopamine loss to be a predictor of cognitive impairment in PD (Bruck et al 2001; Duchesne et al 2002; Holthoff-Detto et al 1997; Marie et al 1999; Muller et al 2000; Rinne et al 2000; van Beilen et al 2008). In regards to dopamine receptor subtypes, the lack of association between cognition and regional D₁ receptors in Study 2 may be due to the relative roles of D₁- and D₂-like receptors in “sustained” and “flexibility” processes, respectively. As discussed in Chapter 6, the tasks performed in Study 2 (Wisconsin card sorting test and Stockings of Cambridge) largely measure cognitive flexibility (although see below), which in turn, has been related to D₂ receptors (see Bilder et al 2004; Cools 2006; Seamans and Yang 2004). In contrast, tasks heavily reliant on sustained representation of a “goal” state (e.g. working memory or sustained attention tasks) are proposed to be preferentially related to cortical D₁ receptors (see Durstewitz and Seamans 2002). Nevertheless, synergistic or cooperative actions between D₁- and D₂-like receptors likely regulate cognitive (including flexibility) processes (Floresco and Magyar 2006; Floresco et al 2006). The correlations in the
current thesis indicate specific dopaminergic mechanisms (i.e. pre-synaptic and intra-synaptic dopamine function) in the performance of cognitive, particularly executive, processes, and add to the growing body of literature that has used molecular imaging techniques to probe dopaminergic correlates of human cognitive function.

While such correlations are promising, there are several limitations that need to be considered. These limitations, in the technique itself, as well as to theoretical or methodological reasons, are common to studies of this nature, and consequently affect our ability to meaningfully interpret the observed relationships. For instance, a major consideration of these studies concerns the specificity of the dopamine/cognition relationships. In light of our, and previous molecular imaging studies reviewed in Chapter 2, it is apparent that delineating regionally specific components of the dopamine system critical for specific cognitive processes is difficult. There are several reasons for this. In part, this is due to the difficulty to refer to specific functions of brain structures, particularly the striatum, in isolation of other regions and circuits. This problem is highlighted in Study 2 of the current thesis. Here, it is unknown whether striatal dopamine depletion in PD impairs set-shifting/executive performance through striatal dopamine loss per se (indicating a specific role for striatal dopamine in set-shifting performance) or indirectly through disruption of the fronto-striato-thalamic loops. Neither of these two interpretations can be discounted using current molecular imaging approaches. Therefore, associations between dopamine markers and cognitive measures need to be considered within the context of regional circuits, in particular the fronto-striato-thalamic loops. Another reason for the reduced specificity of these studies concerns the cognitive tasks used to assess neuropsychological processes. The cognitive tasks employed in previous studies, including those in the current thesis, are complex, consisting of a number of different cognitive processes and presumably engaging multiple regions and subregions. Therefore, delineating the specific cognitive process that is modulated by dopamine is a challenging, if not an unfeasible task. Again the current thesis illustrates this problem. Performance on verbal fluency tasks, as used in Study 1, involves numerous components such as generating words over a set period of time, an intact vocabulary in order to select the words, retrieval of words, verbal memory to keep track of words already generated, and executive processes to coordinate and monitor task performance (Ruff et al 1997). Likewise, while performance on the Wisconsin card sorting task used in Study 2 is purported to mainly
reflect attentional set-shifting, it also involves other processes such as concept formation, cognitive flexibility, abstract reasoning and working memory. Molecular imaging (as opposed to functional imaging), cannot temporally track these different components of the cognitive task at hand, which may be differentially regulated by dopamine function. As such, the observed relationships between dopamine changes (such as regional dopamine release or dopamine loss in the putamen) and task performance (such as verbal fluency and Wisconsin card sorting task), must be interpreted with a degree of caution in respect to the specific cognitive process it is attributed to reflect. Future studies may benefit from assessing simple cognitive processes rather than complex tasks in order to overcome this limitation. Third, the specific relationships detected in previous studies are probably largely influenced by the limited regions assayed and radioligands used. As discussed in Chapter 2, the striatum has been a key focus of previous dopamine/cognition relationships partly because it is a large, high-density structure that is easily visualised and quantified, and because of the limited availability, at least until recently, of radioligands that can adequately measure molecular targets in extrastriatal regions. With the recent development and availability of dopamine radioligands with high affinity, specific to nondisplaceable ratios and selectivity, relationships between dopamine markers and cognition in extrastriatal areas and within mesolimbic and mesocortical dopamine pathways can be assessed. Recent studies using radioligands such as $[^{18}\text{F}]$fallypride, $[^{11}\text{C}]$NNC 112 and $[^{11}\text{C}]$FLB 457 have been successful at detecting relationships between dopamine markers and cognition in various extrastriatal regions such as the thalamus, anterior cingulate, prefrontal and temporal cortex (Aalto et al 2005; Abi-Dargham et al 2002; Christian et al 2006; Lumme et al 2007; Riccardi et al 2006). Using $[^{18}\text{F}]$fallypride, the current thesis also detected significant dopamine/cognition relationships in two extrastriatal regions, the thalamus and substantia nigra. Further development of more dopaminergic radioligands for extrastriatal measurement but also greater selectivity for different components of the dopamine system will aid in the exploration of dopamine’s role in cognition.

A significant drawback of the molecular imaging approach concerns the correlational nature of most studies, such that cognitive function is not measured at the time of the imaging measurement. Therefore, no causative role can be implied, and variables other than those in question may influence the associations. Of note in the current thesis is
that the correlation between $[^{18}\text{F}]$FDOPA $K_i$ and Wisconsin performance in PD was not due to variables such as age or measures of disease severity, although other variables (such as IQ) were not controlled for. Due to challenges associated with Study 1, which was a 4-scan, 2-drug challenge study, variables such as age, IQ, education level and gene variants were not controlled, which may have masked the detection of other relationships. Although admittedly difficult, future studies should attempt to control for such variables, as well as the effects of novelty, task difficulty, and sensory and motor function on cognitive performance and dopamine activation. Related to the correlational approach of ours and previous studies is the importance to control for multiple comparisons, particularly when numerous brain regions and cognitive processes are sampled. Many of the studies reviewed in Chapter 2 did not adequately control for the number of correlations assessed, which increases the risk of a false discovery being made. In the current thesis, we corrected for multiple tests by controlling the false discovery rate, i.e. the expected proportion of falsely rejected hypotheses (Benjamini and Hochberg 1995). This Bonferroni-type procedure is useful for controlling false discoveries but at the same time increasing power when there are multiple comparisons such as in brain imaging and behavioral studies. Future studies may want to consider using this or a similar method for controlling multiple correlations. Lastly, a further, but important consideration when interpreting associations between dopaminergic markers and cognitive measures relates to the functional meaning attached to changes in molecular markers, and the overall meaning of “dopamine function”. As discussed in this and previous chapters of this thesis, the interpretation of the imaging measurement (such as radioligand binding or uptake) is complex, and often confounded by technical and theoretical reasons. For example, post-synaptic receptor radioligands are often confounded by receptor plasticity (up- or down-regulation or denervation supersensitivity) while $D_2$ radioligand displacement studies (intended to measure intra-synaptic dopamine) may be confounded by the pharmacological challenge on the $D_2$ receptors themselves. Until we fully understand what a change in the dopamine imaging parameter actually reflects, and how it relates to the definition of “dopamine function”, the interpretation of such changes with neuropsychological processes will be unclear or incomplete.

In summary, while molecular imaging with PET and SPECT provides a new approach to explore the neurochemical underpinnings of higher cognitive functions it is still in its
infancy, and subject to several limitations that affect the interpretation of observed relationships. While molecular imaging of dopamine and cognition has not, to a large extent, provided original insights to the role of dopamine in cognitive processes, it offers a powerful and unparalleled method to test and validate behavioural models of dopamine function derived from animal or computational studies, in vivo, in living humans. In addition to the investigation of human cognition, correlates of dopaminergic indices such as dopamine release with human emotion may be particularly promising, as humans, in contrast to animals, can describe subjective states under experimental conditions in which dopamine concentration is altered. With further advancement in instrumentation, radioligand development and improvements in study design, molecular imaging will be a promising tool to further test and examine dopaminergic correlates of human cognition, mood and behavior.

7.2.6 Limitations of the current experimental studies

There are several limitations of the experimental studies in this thesis, some of which have been discussed in detail in this, and previous chapters. These include limitations associated with the radioligands, $^{11}$C[NNC 112 and $^{18}$F]FDOPA, in regards to pharmacological selectivity and specificity in the cortex, medication status of PD patients in Study 2, the variability amongst subjects in the time interval between $^{18}$F]fallypride scans and $^{11}$C[NNC 112 and $^{18}$F]FDOPA scans, use of a reference tissue input instead of an arterial input, and limitations associated with the fixed-scan order of Study 1. Most of these limitations are not unique to the current thesis, however, and are mainly a function of the difficulties in recruiting for and conducting such demanding studies. Other limitations, such as the varied interval between PET scans, were largely due to scheduling procedures within the PET centre of the research institute. While it would have been ideal to have a fixed interval between each PET scan of Study 1 and Study 2 (e.g. 2 weeks), the PET centre had to cater for several institutes and research groups, and scheduled each group’s PET requests one month at a time. As such, set days were not allocated for use by a research group or investigators, making it unfeasible to arrange the subject’s PET scans at a fixed interval. In addition, as $^{18}$F]fallypride is a difficult radioligand to synthesize, occasional failed syntheses resulted in repeated $^{18}$F]fallypride scans for some subjects, which increased or varied
the interval between scans. Another limitation of the current thesis which warrants further discussion is the fixed scan order and absence of a placebo in Study 1. This study did not randomise the order of the $^{18}$F-fallypride scans amongst subjects, performing the four scans in a fixed order of baseline 1 (test), baseline 2 (retest), amphetamine and then AMPT. In addition, the study did not include a placebo condition for each of the drug interventions, amphetamine and AMPT. We acknowledge therefore, that the possibility of order effects such as adaptation or habituation cannot be ruled out, although it should be noted that previous studies are also susceptible to order effects as a fixed scan order has been standard in such PET and SPECT studies with amphetamine and AMPT (e.g. Fujita et al 2000; Laruelle et al 1997a; Riccardi et al 2006, 2007; Verhoeff et al 2001). The fixed scan order is particularly problematic for the neuropsychological testing however, as cognitive change scores would have been susceptible to practice effects. Likewise, as a placebo was not given, subjects were not “blind” to the drug intervention, and therefore anticipation of receiving amphetamine or AMPT may have influenced, in particular, the neuropsychological and mood results. Of note however, is that no adequate placebos that mimic the marked physiological effects of amphetamine and in particular, AMPT, exist, which would reduce the effectiveness of including a placebo condition. As previously discussed, we performed the scans in a fixed order because it was not known how long residual effects of amphetamine and AMPT last and also because the primary objective of the study was to examine dopamine levels on $^{18}$F-fallypride binding. Therefore, although a randomized, double-blind placebo-controlled design may have been preferable, particularly to study the neuropsychological effects of drug administrations, it may have limited the ability to answer a simple question of whether $^{18}$F-fallypride binding is affected by dopamine levels. A further limitation common to imaging studies was that we did not control for critical factors such as subject IQ or baseline dopaminergic states, which may have added to the variability in neuropsychological and PET imaging parameters, and influenced the relationships between dopamine (particularly phasic dopamine release) and cognitive function. A reasonably direct assessment of baseline dopamine levels is through the functional polymorphism (Val/Met) in the COMT gene and through imaging of D$_2$ receptor occupancy following endogenous dopamine depletion with PET and SPECT. However, assessment of genetic polymorphisms is a resource demanding procedure and requires large study samples, while our study with $^{18}$F-fallypride and AMPT (Study 1) failed to increase radioligand binding, and thus provide a measure of
baseline dopamine levels. Therefore, while control of such critical factors would be preferable, often it is unfeasible or the methodology does not allow us to reliably do so. As with all experimental studies, the results of this thesis need to be interpreted within the context of these limitations, bearing in mind the challenges associated with conducting such studies and the primary objectives of this thesis.

7.2.7 Relevance and implications of the current findings

This thesis has provided information on the feasibility and capability of three dopamine radioligands, \([^{18}F]fallypride\), \([^{11}C]NNC 112\) and \([^{18}F]FDOPA\), to assess different components of the dopamine system in striatal and extrastriatal regions. Knowledge of the suitability and behavior of these radioligands within certain imaging paradigms will undoubtedly be of assistance for designing studies to evaluate the dopamine system in a variety of psychiatric and neurological disorders. For instance, the question of whether the \textit{in vivo} binding of \([^{18}F]fallypride\) is vulnerable to endogenous competition by dopamine in humans has wide-ranging implications for the study of striatal and extrastriatal dopamine transmission in diseases such as schizophrenia, PD and drug addiction. On the basis of the current findings, \([^{18}F]fallypride\) will be useful for examining regional phasic dopamine transmission in areas such as the striatal subdivisions, substantia nigra, temporal cortex and orbitofrontal cortex, whereas it may not be useful for reliably measuring baseline or tonic dopamine transmission. Likewise, the D\(_1\) radioligand, \([^{11}C]NNC 112\), may hold some promise for examining the regional (subcortical and cortical) distribution and density of post-synaptic D\(_1\)-like receptors in human psychiatric disorders, although, on the basis of a recent finding demonstrating the relatively high selectivity of \([^{11}C]NNC 112\) for 5-HT\(_{2A}\) receptors (Slifstein et al 2007), it may be more applicable for examining the striatum only, or extrastriatal regions with low to intermediate concentrations of 5-HT\(_{2A}\) receptors such as the thalamus, hippocampus, brainstem and lamina V of the neocortex (Pazos et al 1985). At the very least caution must be expressed due to the added noise from cortical 5-HT\(_{2A}\) receptor binding. On the basis of our findings and in light of previous \textit{in vitro} and \textit{in vivo} studies of D\(_1\) receptor binding in PD, evidence suggests that D\(_1\) receptors are not altered, at least in non-demented PD, and consequently therapeutic targets for the D\(_1\) receptor in PD may be of limited benefit. Based on the current work, D\(_1\) receptors may
also have little predictive value for frontostriatal cognitive function in PD, at least for the processes measured in this thesis (set-shifting and planning) which largely involve behavioral flexibility. Nevertheless, the relevance of these findings is restricted due to our failure to observe overall cognitive impairment in our sample. Given the consistent findings implicating a critical role for optimal PFC D₁ receptor stimulation for working memory performance in non-human primates (Arnsten et al 1994; Cai and Arnsten 1997; Sawaguchi and Goldman-Rakic 1991; Sawaguchi and Goldman-Rakic 1994; Williams and Goldman-Rakic 1995), and a previous PET imaging study with [¹¹C]NNC 112 linking PFC D₁ receptor availability with working memory impairments in schizophrenia (Abi-Dargham et al 2002), our results do not rule out a role for D₁ receptors in executive processes but may point to a specificity of function of D₁ receptors for working memory, and in particular the “maintenance” or “holding” components of working memory, as assessed by delayed-response tasks used in the non-primate literature. In contrast, D₂ receptors may play a role in the frontostriatal deficits related to cognitive flexibility, as discussed above. Alternatively, our findings may suggest non-dopaminergic mechanisms in the cognitive profile of non-demented PD.

Our proposal that [¹⁸F]FDOPA is not a suitable marker for cortical pre-synaptic dopamine synthesis has important implications within the field of molecular imaging. Of particular relevance is the quantification of cortical [¹⁸F]FDOPA uptake with the Patlak model using a reference tissue input (Patlak and Blasberg 1985). Because of the likely model violation, this method of analysis for cortical uptake should be strongly discouraged in future studies. If careful evaluation of cortical [¹⁸F]FDOPA decarboxylation with arterial-input compartmental models (e.g. Gjedde et al 1991; Kuwabara et al 1993) confirms our assertion that the cortical [¹⁸F]FDOPA PET signal is not specific, future research groups may question using this radioligand for assessing cortical dopamine synthesis and storage. Furthermore, the current need for selective radioligands for pre-synaptic measurement of cortical dopamine function may well drive development of more specific tracers. Our current findings regarding the non-specific nature of cortical [¹⁸F]FDOPA adds a layer of complexity to previous published studies that have reported increases or decreases of cortical dopamine synthesis in PD using [¹⁸F]FDOPA with reference tissue inputs (Bruck et al 2005; Ito et al 2002; Kaasinen et al 2001; Rakshi et al 1999; Rinne et al 2000). As such, the functional interpretation of these previously published results in PD may have to be revisited. The
findings relating to $[^{18}\text{F}]$FDOPA quantification has been accepted for publication in a peer-reviewed journal (Psychiatry Research: Neuroimaging) (see Appendix 3 for copy of proof) which should aid in alerting the scientific community to the shortcomings of cortical $[^{18}\text{F}]$FDOPA measurement.

7.2.8 Future research directions

As mentioned above, PET imaging of selected dopamine targets using $[^{18}\text{F}]$fallypride, $[^{11}\text{C}]$NNC 112 and $[^{18}\text{F}]$FDOPA combined with novel dopamine imaging techniques should be used to further our understanding of various psychiatric and neurological disorders implicating dopamine in its pathophysiology, such as schizophrenia, PD and substance abuse or addiction. In particular, displacement paradigms with $[^{18}\text{F}]$fallypride should be extended from healthy, to clinical, particularly schizophrenic, populations. For instance, the dopamine hypothesis of schizophrenia postulates that there is a cortical/subcortical dopaminergic imbalance, such that an excess of dopamine subcortically is related to positive symptoms, while a deficit of dopamine cortically is related to the negative and cognitive symptoms of the illness (Abi-Dargham 2004; Weinberger 1987). A second hypothesis postulates an imbalance between baseline tonic and phasic dopamine transmission, such that low basal levels of dopamine predisposes to excessive phasic release of dopamine (Grace 1993). On the basis of our findings in healthy controls, $[^{18}\text{F}]$fallypride may be useful for investigating the former hypothesis, but not the latter, due to the failure of $[^{18}\text{F}]$fallypride to be modulated by transient dopamine depletion and subsequently measure tonic dopamine levels. Previous imaging studies, using the D$_2$ radioligands $[^{11}\text{C}]$raclopride and $[^{123}\text{I}]$IBZM, which allow for measurement in only the striatum, have observed increased amphetamine-induced phasic dopamine release in the striatum of schizophrenia patients (Abi-Dargham et al 1998; Breier et al 1997; Laruelle et al 1999; Laruelle et al 1996), which was associated with an increase in positive symptoms (Laruelle et al 1999). Future studies should consider using $[^{18}\text{F}]$fallypride and amphetamine challenge to assess regional abnormalities (subcortical and cortical) of phasic dopamine release in schizophrenia, and to compare whether alterations of phasic dopamine transmission differs in striatal and extrastriatal regions. Based on the above hypothesis, elevated dopamine release would be predicted in the striatum, while blunted dopamine release would be predicted
in cortex, such as the orbitofrontal cortex and other extrastriatal regions.

[^18F]Fallypride binding competition techniques should also be considered in other neuropsychiatric disorders (e.g. PD or drug addiction) and using other pharmacological challenges such as L-dopa. Previous PET studies in PD have reported L-dopa-induced displacement of [¹¹C]raclopride binding in the striatum (Pavese et al 2006; Tedroff et al 1996b), and its correlation with L-dopa-induced clinical (i.e. motor) improvement (Pavese et al 2006). Broadly speaking, future studies using[^18F]fallypride with L-dopa challenge could examine the cognitive correlates of L-dopa-induced synaptic dopamine release in striatal and extrastriatal regions in early to advanced PD. Studies of this nature in PD should attempt to formulate predictions according to the known spatiotemporal progression of dopamine depletion (dorsal to ventral) within the fronto-striatal circuitries in PD, the parallel cognitive profile of this dopamine depletion progression, the underlying task demands of specific cognitive tests and the basal level of dopamine within distinct neural circuits as a function of disease stage (see Cools 2006). Furthermore, future studies may also want to consider the extent of dopaminergic (striatal and extrastriatal) alteration in PD with dementia (including D₁ receptors), as the co-morbidity of dementia with PD may involve separate or additional striatal and extrastriatal dopaminergic mechanisms, or alternatively, non-dopaminergic mechanisms, in its pathology.

More broadly, future studies should consider further examining the interactions between components of the dopamine system and other neurotransmitters such as glutamate, GABA and serotonin that may affect dopamine-mediated responses in the human brain. Interactions between dopamine, GABA and acetylcholine systems have been pioneered by Dewey and colleagues using in vivo competition binding techniques (e.g. Dewey et al 1995; Dewey et al 1993; Dewey et al 1992). Likewise, stimulant-induced dopamine release has been shown to be regulated by glutamate in several imaging studies (Kegeles et al 2000; van Berckel et al 2006), such that amphetamine-induced dopamine release is enhanced by glutamate blockade, presumably by impairing the glutamate inhibitory pathway that acts to protect against excess of endogenous dopamine (e.g. Carlsson et al 1999). Such dopamine-glutamate interactions have important implications to the study of schizophrenia, as abnormalities in glutamate transmission has been proposed in this disorder (Carlsson et al 1999; Moghaddam 2003). Future studies of
dopamine imaging may also benefit from conducting multiple tracer (and imaging modality) studies in the same subject, in order to further elucidate interactions between pre- and post-synaptic dopaminergic markers, receptor subtypes (e.g. D₁ and D₂), regional actions (e.g. striatal versus extrastriatal) and functional brain responses to changes in dopamine (e.g. fMRI and receptor occupancy) at an individual level, and the alteration of such interactions in pathology or their role in cognitive processes. Several studies have adopted this multi-tracer or modality approach (e.g. Eidelberg et al 1990; Meyer-Lindenberg et al 2005; Meyer-Lindenberg et al 2002; Ouchi et al 1999a; Yang et al 2004b), including Study 2 of the current thesis.

An exciting and recent development in neuroimaging research has been to image the influence of genes that affect dopaminergic signaling in the brain (see Mattay and Goldberg 2004). A promising candidate is the gene for COMT, an enzyme important for dopamine catabolism and for regulating cortical dopamine flux (Chen et al 2004). The COMT gene contains an evolutionarily recent mutation that substitutes methionine (met) for valine (val) at codon 108/158 (Val₁₀₈/₁₅₈ Met), which results in significantly lower activity of the enzyme in the brain (Lotta et al 1995). The lower activity of the met allele is predicted to lead to greater dopamine levels in the PFC. This functional polymorphism in the COMT gene has been shown to influence PFC activation and PFC-dependent neuropsychological function (Egan et al 2001), individual variation in the brain response to amphetamine (Mattay et al 2003), and prefrontal-midbrain interaction and modulation of midbrain dopamine synthesis (Meyer-Lindenberg et al 2005). Met allele carriers (who presumably have greater prefrontal dopamine tone at baseline) were reported to perform superior to val allele carriers on an executive cognition task and show a more efficient physiological response in the PFC (Egan et al 2001), but were adversely affected by amphetamine, such that prefrontal efficiency and executive performance were diminished following the stimulant, postulated to be due to increased dopamine signaling that pushed these individuals beyond the critical threshold on the inverted ‘U’ functional response curve (Mattay et al 2003). Using multi-modal imaging, the effect of COMT genotype on a measure of dopaminergic function (midbrain [¹⁸F]FOPDA uptake) and its interaction with prefrontal activity was explored (Meyer-Lindenberg et al 2005). Met homozygotes predicted reduced dopamine synthesis in the midbrain, which in turn was predicted by lower working memory-induced PFC activation, but higher task-unrelated PFC activity. Task-related and task-
unrelated activity were also oppositely related to variations in midbrain dopamine in the val group, providing in vivo evidence of a dopaminergic tuning mechanism in PFC (Meyer-Lindenberg et al 2005). Importantly, these exciting results demonstrate the potential for neuroimaging techniques, both functional and molecular, to provide a sensitive assay to explore the impact of genetic variability on human brain function. The effect of the COMT polymorphism, as well as other genetic polymorphisms, on regional dopamine levels (both phasic and tonic as measured by competition binding techniques), PFC D1 receptor availability and other markers of brain dopaminergic function, will undoubtedly play a major focus in future molecular imaging studies.

Finally, as has been illustrated throughout this thesis, the advancement and efficacy of molecular imaging to comprehensively measure the dopamine system in vivo, is largely reliant on the development of more selective radioligands. In fact, a major limitation of molecular imaging for its expanded use in neuroscience in general is the paucity of available tracers. The current thesis demonstrated the need for more specific radioligands for pre-synaptic dopamine synthesis in the cortex (Study 2), and although unknown at the outset of this thesis, recent studies have highlighted the need for more selective radioligands for D1 receptors, also in cortex (see above and discussion in Study 2). Development of an array of selective radioligands for evaluating dopamine components, receptor subtypes (D1,D2,D3,D4,D5), extrastriatal regions, synaptic neurotransmission, receptor sub-populations (e.g. G-protein coupled versus uncoupled forms), and second messenger systems, along with radioligands with a range of kinetic profiles, will greatly advance our ability to measure precise aspects of the dopamine system and consequently its expression in pathology and mechanisms for regulating cognitive and behavioral processes. Some progress has been made towards these goals. For instance, comparison of D2 agonists such as [11C]MNPA or [11C]NPA, which bind to the G-protein coupled form of the receptor, with D2 antagonists such as [11C]raclopride, which bind to both G-protein coupled and uncoupled forms of the receptor (see Narendran et al 2005b), may be used to measure the functional state of receptors. The use of D2 agonists will further advance our capability to image synaptic dopamine neurotransmission, as agonists are more sensitive than antagonists to displacement by endogenous dopamine (Narendran et al 2004; Seneca et al 2006). This will aid in detecting small changes in synaptic dopamine, which may be useful for studies that stimulate dopamine with less potent means, e.g. with behavioural or
cognitive paradigms. Nevertheless, more radioligands are required for future use. Several research centers, such as the NIMH Molecular Imaging Branch, have been established specifically with the goal to discover and evaluate new radioligands for the brain. Further, the increasing collaboration between academia and pharmaceutical industries will undoubtedly assist in radioligand development, including those targeted for the dopamine system.

7.2.9 Concluding remarks

This thesis, by means of PET technology and three dopamine radioligands, $[^{18}\text{F}]$fallypride, $[^{11}\text{C}]$NNC 112 and $[^{18}\text{F}]$FDOPA, assessed various components of the dopamine system in striatal and extrastriatal regions in normal and PD brain and their relationship to executive cognitive function. Several key findings were reported. The D$_2$-like radioligand, $[^{18}\text{F}]$fallypride, was demonstrated to be vulnerable to increases in endogenous dopamine induced by oral amphetamine in both striatal and extrastriatal regions in healthy humans, thus providing a promising method to examine alterations in regional phasic dopamine release in neuropsychiatric disorders such as schizophrenia. In contrast, $[^{18}\text{F}]$fallypride was not vulnerable to rapid dopamine depletion induced by AMPT, a puzzling finding that requires further confirmation but nevertheless indicates a limitation of this tracer to be extended to psychiatric disorders for baseline dopamine measurement. In a disorder characterized by extensive dopamine depletion (PD), D$_1$ receptors were not altered in subcortex or cortex, as measured by the D$_1$-like receptor radioligand, $[^{11}\text{C}]$NNC 112, and were not related to frontostriatal cognitive processes. The findings of this thesis question the measurement of $[^{18}\text{F}]$FDOPA uptake in cortex and the suitability of this tracer as a marker of cortical dopamine synthesis, based on the observation of paradoxically greater $[^{18}\text{F}]$FDOPA uptake in white than adjacent gray matter and the likely violation of the reference tissue input model.

Several correlations between dopaminergic parameters (phasic dopamine release and striatal dopamine synthesis) and cognitive processes were observed, demonstrating some utility for this technique to explore the dopaminergic correlates of human cognitive function. Nevertheless, limitations common to studies of this nature, including those relating to study design, specificity of the radioligands and tasks, and theoretical
reasons, hinder, at present, the extent to which meaningful interpretations can be made.

In summary, the findings of this thesis indicate promise for \([^{18}\text{F}]\text{fallypride}\) to estimate regional phasic dopamine levels, some promise for \([^{11}\text{C}]\text{NNC 112}\) to measure \(D_1\) receptor availability but demonstrate deficiencies in the cortical \([^{18}\text{F}]\text{FDOPA}\) signal. These findings emphasise the need for more selective radioligands and highlight the complexities of \textit{in vivo} radioligand measurement. With further advancements in radioligand development, instrumentation and study design, PET will enable more precise assessments of the human dopamine system to be made, and will allow investigation of the functional (e.g. cognitive) and clinical consequences of perturbations to this system.
# References


Small effect of dopamine release and no effect of dopamine depletion on
schizophrenics: evidence for a selective increase in dopamine D2 receptors.
living brain from dynamic positron emission tomograms. Synapse 29:37-61.
Kinetics of in vitro decarboxylation and the in vivo metabolism of 2-18F- and 6-
Dagher A, Owen AM, Boecker H, Brooks DJ (2001): The role of the striatum and
hippocampus in planning: a PET activation study in Parkinson's disease. Brain
124:1020-1032.
Dahlstrom A, Fuxe K (1964): Evidence for the existence of monoamine-containing
neurons in the central nervous system. I. Demonstration of monoamines in the
C, et al (1997): Presynaptic dopaminergic function in the striatum of
Davis KL, Kahn RS, Ko G, Davidson M (1991): Dopamine in schizophrenia: a review
Initial human PET imaging studies with the dopamine transporter ligand 18F-
de Jong HW, van Velden FH, Kloet RW, Buijs FL, Boellaard R, Lammertsma AA
Autoradiographic localization of D1 and D2 dopamine receptors in the human


Vanessa L Cropley  PhD Thesis


Mehta MA, Gumaste D, Montgomery AJ, McTavish SF, Grasby PM (2005): The effects of acute tyrosine and phenylalanine depletion on spatial working memory and planning in healthy volunteers are predicted by changes in striatal dopamine levels. *Psychopharmacology (Berl)* 180:654-663.


Montagu KA (1957): Catechol compounds in rat tissues and in brains of different animals. *Nature* 180:244-245.


Reeves SJ, Grasby PM, Howard RJ, Bantick RA, Asselin MC, Mehta MA (2005): A positron emission tomography (PET) investigation of the role of striatal


Yang YK, Yu L, Yeh TL, Chiu NT, Chen PS, Lee IH (2004b): Associated alterations of striatal dopamine D2/D3 receptor and transporter binding in drug-naive patients.


9 Appendices
APPENDIX 1: Reprint of Cropley et al. 2006a (Biological Psychiatry)
Molecular Imaging of the Dopaminergic System and its Association with Human Cognitive Function

Vanessa L. Cropley, Masahiro Fujita, Robert B. Innis, and Pradeep J. Nathan

Molecular imaging with positron emission tomography (PET) and single photon emission computed tomography (SPECT) has recently been used to examine dopamine (DA) function and its relationship with cognition in human subjects. This article will review PET and SPECT studies that have explored the relationship between cognitive processes and components of the DA system (pre-, intra-, and postsynaptic) in healthy and patient populations such as Parkinson’s disease (PD), schizophrenia, Huntington’s disease, and aging. It is demonstrated that DA activity modulates a range of frontal executive-type cognitive processes such as working memory, attentional functioning, and sequential organization, and alterations of DA within the fronto–striato–thalamic circuits might contribute to the cognitive impairments observed in PD, schizophrenia, and normal aging. Although associations between DA and cognitive measures need to be considered within the context of fronto–striato–thalamic circuitry, it is suggested that striatal (especially caudate) DA activity, particularly via D2 receptors, might be important for response inhibition, temporal organization of material, and motor performance, whereas cortical DA transmission via D1 receptors might be important for maintaining and representing on-going behavior.

Key Words: PET, SPECT, dopamine, cognition, Parkinson’s disease, schizophrenia

Since the discovery of dopamine (DA) in the central nervous system almost 50 years ago (Carlsson et al 1958), considerable research has implicated the DAergic system as a modulator of cognitive processes, particularly those understood to be subserved by the frontal cortex, striatum, and associative structures. Certainly one of the most established findings is its role in working memory. Pioneering work by Goldman-Rakic and colleagues reported that depletion of DA by 6-hydroxydopamine in the area surrounding the principal sulcus impaired performance on a delayed alternation task of working memory in monkeys, mirroring deficits produced by surgical ablation to the same area (Brozoski et al 1979). This deficit was pharmacologically reversed by the DA agonists levodopa (L-Dopa) or apomorphine. Subsequent studies in monkeys and rodents have demonstrated the importance of mesocortical and striatal DAergic inputs in working memory and higher order cognitive processes (Arnsten 1998; Watanabe et al 1997; Young et al 1998).

Evidence of a role of DA in human cognitive processes has been provided by pharmacological challenge and imaging studies. Reduction in DA transmission via DAergic antagonists and depletion of the DA precursors tyrosine and phenylalanine impaired performance (Harmer et al 2001; Mehta et al 1999), whereas low doses of DAergic agonists improved performance (Luciana et al 1998; Roesch-Ely et al 2005), in executive tasks such as working memory, planning, and attentional set-shifting. Studies of regional cerebral blood flow after DA stimulants in healthy subjects have also delineated particular working memory–related brain circuits that are modulated by DA (Mattay et al 2000; Mehta et al 2000); however, a limitation of these studies is their inability to directly examine DAergic correlates of cognition. Knowledge of the latter is important in determining the anatomical regions that are critical to cognitive processes modulated by the DA system.

Molecular imaging with positron emission tomography (PET) and single photon emission computed tomography (SPECT) allows the direct assessment of brain neurochemical systems by using biochemical markers of neurotransmitters, transporters, or enzymes. Thus, it can directly measure components of the DA system in living human subjects, providing information that could only previously be assessed in animals, cell cultures, and postmortem brains. Because of the large number of radiotracers that can examine pre-, intra-, and postsynaptic components of the DA system and because of the strong experimental and observational evidence linking DA and cognition, molecular imaging techniques have recently examined relationships between molecular markers of the DAergic system and cognitive processes in normal subjects and patient populations. This review describes molecular imaging studies that have explored the relationship between cognitive processes and components of the DA system in healthy human subjects and several patient groups with known or hypothetical DA and cognitive dysfunction, such as Parkinson’s disease (PD), schizophrenia, and Huntington’s disease.

Assessment of Pre-, Intra-, and Postsynaptic Components of the DA System. Molecular imaging with PET and SPECT is a nuclear medicine technique that uses radiopharmaceuticals to image the regional distribution and kinetics of chemical compounds within the brain. A wide range of PET and SPECT radiotracers have become available that can assess presynaptic, postsynaptic, and intrasynaptic components of the DA system (see Figure 1). Presynaptic markers of DA neurons include [18F]FDOPA and radioligands of the DA transporter (DAT). Postsynaptic components of the DA system are assessed with a variety of D1 and D2 receptor family radioligands. For estimation of intrasynaptic components of the DA system, competition for receptor binding between certain D2 radioligands and endogenous DA has been assessed (Laruelle 2000).

Examination of Striatal Versus Extrastriatal Regions. In early years, molecular imaging studies of the DA system were largely limited to the striatum. Since the mid 1990s, however, the development of DA receptor radioligands with higher affinity and specificity to non-displaceable ratios, together with improvements in scanner sensitivity, has allowed extrastriatal as well as striatal regions to be measured. Several PET D2 antagonist

From the Department of Physiology (VLC, PJN), Behavioural Neuroscience Laboratory, Monash Centre for Brain and Behaviour, Monash University, Victoria, Australia; and the Molecular Imaging Branch (VLC, MF, RBI), National Institute of Mental Health, Bethesda, Maryland.

Address reprint requests to Vanessa L. Cropley, Behavioural Neuroscience Laboratory, Department of Physiology, Monash Centre for Brain and Behaviour, Monash University, Victoria 3800, Australia; E-mail: cropleyv@mail.nih.gov.

Received June 24, 2005; revised October 7, 2005; accepted March 6, 2006.

doi:10.1016/j.biopsych.2006.03.004 © 2006 Society of Biological Psychiatry
radioligands, such as \[^{18}\text{F}]\text{FDOPA}\) and \[^{11}\text{C}]\text{FLB-457}\), can image and quantify low-density regions such as the thalamus, amygdala, and medial temporal cortices (Farde et al. 1997; Mukherjee et al. 2005). The D\(_1\) receptor radioligand \[^{11}\text{C}]\text{NNC 112}\) also provides a cortical signal superior to older D\(_1\) ligands such as \[^{11}\text{C}]\text{SCH-23390}\) (Hallidin et al. 1998). The ability of molecular imaging to quantify DA parameters in extrastriatal regions is significant to the study of DAergic inputs in cognition.

**Examination of Smaller Brain Regions and Subdivisions.** Improvements in spatial resolution and sensitivity now allow smaller brain regions to be visualized, such as striatal subdivisions and brain stem structures like the substantia nigra. Quantification of smaller subregions is important, because many brain structures are functionally and neurochemically heterogeneous. Recent PET studies have segregated the striatum into several subdivisions, such as the ventral and dorsal striata, and further subdivisions of the caudate and putamen (e.g., Mawlawi et al. 2001). This has served as a basis for examining the proposed functional segregation of the neuronal loops connecting the frontal cortex, thalamus, and striatum (known as frontostriatal or fronto–striato–thalamic circuitry circuits) into “motor,” “limbic,” and “associative” domains (Alexander and Crutcher 1990) and to differentially examine the DA pathways.

**Presynaptic DAergic Modulation of Cognitive Function**

**Striatal \[^{18}\text{F}]\text{FDOPA Studies in Parkinson's Disease.}** \[^{18}\text{F}]\text{FDOPA}\) PET is a useful tool to quantify the loss of nigrostriatal DA terminal function in PD and has been used to monitor disease severity and progression, detect preclinical disease states, and differentiate idiopathic PD from other parkinsonian syndromes (for review, see Heiss and Hilker 2004); however, the clinical manifestations of PD are not only restricted to motor deterioration. Cognitive impairment, usually described as subsylvial-frontal in nature, and dementia are common in PD (Aarsland et al. 2003; Zgaljardic et al. 2003). The capability of \[^{18}\text{F}]\text{FDOPA}\) PET to measure the loss of presynaptic DA terminals places it in a prime position to investigate the cognitive consequences of DA deficiency.

Several studies have directly assessed the relationship between DAergic denervation and cognitive performance in PD with \[^{18}\text{F}]\text{FDOPA}\) (see Table 1). Many of these studies also tested the assertion that the putamen is more important for motor function, whereas the caudate nucleus is more important for behavioral, including cognitive, functions (Alexander and Crutcher 1990). To support this, correlations have been reported between reduced \[^{18}\text{F}]\text{FDOPA}\) uptake in the caudate nucleus and cognitive dysfunction. \[^{18}\text{F}]\text{FDOPA}\) reductions were associated with impaired verbal episodic memory in advanced PD (Holthoff-Detto et al. 1997) and twins discordant for PD (Holthoff et al. 1994), with impairment in a tactile object discrimination task in non-demented, medication withdrawn PD (Weder et al. 1999) and with impairment in attentional and inhibitory functioning (Bruck et al. 2001; Rinne et al. 2000). These findings are consistent with reports of explicit memory and executive deficits in PD (Dubois and Pillon 1997) and the demonstration that executive dysfunction in PD is accompanied by reduced activity within the caudate nucleus and prefrontal regions (Lewis et al. 2003). Such associations between presynaptic DA denervation and certain executive deficits suggest that impaired caudate DAergic transmission might contribute to the cognitive deficits of patients. With the exception of one study (Holthoff et al. 1994), \[^{18}\text{F}]\text{FDOPA}\) reductions in the putamen were associated with only motor disability and not cognitive impairment; however, contrary to these reports and to the caudate nucleus-cognition model, three studies have failed to show an association between caudate nucleus \[^{18}\text{F}]\text{FDOPA}\) uptake and cognitive performance in non-demented PD patients (Broussolle et al. 1999; Bruck et al. 2005; Nagano-Saito et al. 2004). Differences in the clinical features of the PD patients examined (in disease severity, dementia, and so forth) and the cognitive tests used make comparisons amongst studies difficult (see following).

**Extrastrial \[^{18}\text{F}]\text{FDOPA Studies in Parkinson's Disease.}** Disruptions in the mesocorticolimbic pathways have also been associated with cognitive deficits in PD. For instance, Rinne et al. (2000) found an association between reduced \[^{18}\text{F}]\text{FDOPA}\) uptake in the frontal cortex and deficits in working and immediate memory and executive strategies; however, after statistically controlling for disease duration and severity, some of these relationships failed to reach significance. Several subjects also showed mild cortical atrophy, which could potentially account for the lower \[^{18}\text{F}]\text{FDOPA}\) uptake in the frontal cortex and cognitive deterioration. In another study, \[^{18}\text{F}]\text{FDOPA}\) reductions in the anterior cingulate were observed in PD patients with dementia (Ito et al. 2002), although, because aromatic L-amino acid decarboxylase (AADC) is also present in noradrenergic and

---

**Figure 1.** Radioligand target sites and examples of radioligands used for imaging the dopaminergic system with positron emission tomography and single photon emission computed tomography. DA, dopamine.
serotonergic neurons (Tison et al 1991), the cortical [18F]FDOPA reductions might be a composite of DAergic, serotonergic, and noradrenergic systems, which also degenerate in advanced PD.

In contrast, early, non-demented and non-medicated PD patients were recently reported to show increased [18F]FDOPA uptake in cortical areas covering the dorsolateral prefrontal cortex (DLPFC), anterior cingulate, and medial frontal cortex (Bruck et al 2005), replicating previous observations of increased cortical [18F]FDOPA uptake in early, medicated (Rakshi et al 1999), and unmedicated (Kaasinen et al 2001) PD patients. Although increased cortical [18F]FDOPA uptake is counterintuitive, it might be a compensatory process in the cortical–subcortical DA loops (see following). Interestingly, these cortical [18F]FDOPA increases were differentially associated with attentional functioning. In a region encompassing the anterior cingulate and medial frontal cortex, increased uptake was strongly related to decreased Stroop interference time (reflecting decreased processing time to suppress attention), whereas in the right DLPFC, [18F]FDOPA uptake was positively correlated with reaction time on a vigilance test assessing sustained attention. These results are in accordance with blood flow studies linking sustained attention to the right DLPFC (Cabeza and Nyberg 2000) and suppressed attention to the dorsal anterior cingulate and inferior frontal sulcus (Duncan and Owen 2000). Indeed, greater [18F]FDOPA uptake in the dorsal anterior cingulate has recently been associated with reduced interference on the Stroop task in medicated male schizophrenic patients and control subjects (McGowan et al 2004), providing further support for the involvement of anterior cingulate DA function in suppressed attention/response inhibition processes. It is not clear, however, why greater DLPFC [18F]FDOPA uptake was associated with poorer sustained attention. A possible explanation could be that the effect of DA on cognitive performance might be dependant on the nature of the cognitive task (i.e., reflecting specific task

Table 1. Molecular Imaging Studies Showing Associations Between Presynaptic Dopamine and Cognition in Healthy Subjects and Patient Populations

<table>
<thead>
<tr>
<th>Author</th>
<th>Target</th>
<th>Tracer</th>
<th>Population</th>
<th>Cognitive Task(s)</th>
<th>Brain Region</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Holthoff et al 1994</td>
<td>AADC</td>
<td>[18F]FDOPA</td>
<td>PD</td>
<td>Selective reminding task</td>
<td>Caudate and putamen</td>
<td>Reduced uptake associated with worse performance</td>
</tr>
<tr>
<td>Holthoff-Detto et al 1997</td>
<td>AADC</td>
<td>[18F]FDOPA</td>
<td>PD</td>
<td>Selective reminding task (delayed recall)</td>
<td>Caudate</td>
<td>Reduced uptake associated with worse performance</td>
</tr>
<tr>
<td>Weder et al 1999</td>
<td>AADC</td>
<td>[18F]FDOPA</td>
<td>PD</td>
<td>Tactile discrimination task</td>
<td>Caudate</td>
<td>Reduced uptake associated with worse performance</td>
</tr>
<tr>
<td>Rinne et al 2000</td>
<td>AADC</td>
<td>[18F]FDOPA</td>
<td>PD</td>
<td>Stroop</td>
<td>Frontal cortex</td>
<td>Digits span (backwards), verbal fluency, verbal immediate recall</td>
</tr>
<tr>
<td>Bruck et al 2001</td>
<td>AADC</td>
<td>[18F]FDOPA</td>
<td>PD</td>
<td>Stroop</td>
<td>Caudate</td>
<td>Reduced uptake associated with worse performance</td>
</tr>
<tr>
<td>Nagano-Saito et al 2004</td>
<td>AADC</td>
<td>[18F]FDOPA</td>
<td>PD</td>
<td>RCPM</td>
<td>Left hippocampus</td>
<td>Positive correlation between uptake and RCPM</td>
</tr>
<tr>
<td>McGowan et al 2004</td>
<td>AADC</td>
<td>[18F]FDOPA</td>
<td>Schizophrenia</td>
<td>Stroop</td>
<td>Dorsal AC</td>
<td>Correlation between uptake and performance in patients and controls</td>
</tr>
<tr>
<td>Bruck et al 2005</td>
<td>AADC</td>
<td>[18F]FDOPA</td>
<td>PD</td>
<td>Stroop, Vigilance test</td>
<td>AC-MFC</td>
<td>Increased uptake associated with better performance</td>
</tr>
<tr>
<td>Muller et al 2000</td>
<td>DAT</td>
<td>[123I]CIT</td>
<td>PD</td>
<td>Card sorting test, digit ordering test, logical memory</td>
<td>Caudate and putamen</td>
<td>Reduced binding associated with worse performance</td>
</tr>
<tr>
<td>Mozley et al 2001</td>
<td>DAT</td>
<td>[99mTc] TRODAT-I</td>
<td>Healthy</td>
<td>Pennsylvania verbal learning test, Stroop</td>
<td>Caudate and putamen</td>
<td>Better performance associated with higher binding</td>
</tr>
<tr>
<td>Duchesne et al 2002</td>
<td>DAT</td>
<td>[123I]CIT</td>
<td>PD</td>
<td>Simultaneous processing task</td>
<td>Left striatum</td>
<td>Lower binding associated with longer processing</td>
</tr>
</tbody>
</table>

AADC, aromatic acid decarboxylase; PD, Parkinson’s disease patients; RCPM, Raven’s Colored Progressive Matrics; AC, anterior cingulate; AC-MFC, anterior cingulate and medial frontal cortex; DLPFC, dorsolateral prefrontal cortex; DAT, dopamine transporter; RT, reaction time; R-OCF, Rey-Osterrieth’s Complex Figure; WM, working memory; COAT, Controlled Oral Association Test; WAIS-R, Wechsler Adult Intelligence Scale-Revised.

www.sobp.org/journal
demands) and the basal level of DA in the underlying fronto–striato–thalamic circuitry, as has been proposed by others (e.g., Cools et al 2003).

Finally, [18F]FDOPA uptake in only the left hippocampus was positively correlated with Raven’s Colored Progressive Matrices (RCPM), a test reliant on executive and visuospatial functions, in non-demented PD patients (Nagano-Saito et al 2004). It was speculated that the involvement of hippocampal DA in RCPM might have represented recruitment of an alternative network to compensate for caudate nucleus dysfunction, a concept supported by a PET activation study in PD patients demonstrating decreased caudate nucleus activation but increased hippocampal activation during a planning task (Dagher et al 2001); however, it should be noted that, because the [18F]FDOPA signal-to-noise ratio is quite low in cortex, it is uncertain whether [18F]FDOPA uptake can be reliably quantified in cortical areas. Therefore, the aforementioned studies should be viewed as preliminary.

**DAT Studies in Parkinson’s Disease.** Studies that have measured striatal DAT binding in PD and cognitive function also support an association between presynaptic DA hypofunction and impairment on executive-type processes (see Table 1). In non-demented, medicated PD patients, decreased [18F]Cromifensine binding in the right caudate nucleus was associated with impairments on the object alternation task, which assesses attentional set-shifting, whereas a weaker, negative correlation in the bilateral putamen was observed between [123I]Cromifensine binding and performance on a planning task (Marie et al 1999). The object alternation findings indicate that loss of caudate DA terminal function is associated with impaired attentional set-shifting. In the opposite direction, Roberts et al (1994) reported that higher DA release in the monkey caudate nucleus was associated with better set-shifting performance, further suggesting a role of caudate DA in set-shifting processes. With 2β-carbomethoxy-3β(4-iodophenyl) tropane ([123I]β-CIT) in non-demented PD, decreased DAT uptake in both the caudate nucleus and putamen was associated with impaired performance on the modified card sort test and digit-ordering, representing abilities such as attentional set-shifting, cognitive flexibility, and working memory as well as immediate and delayed recall on the logical memory subtest (Muller et al 2000), although these relationships were relatively weak and might have been a function of age and motor disability. Furthermore, in non-demented PD, deficits in simultaneous processing time (a type of attentional processing demanding a sharing of attention) in unmedicated (“off”) states were associated with lower [123I]β-CIT binding in the left striatum, suggesting that deficits in cognitive tasks that involve a sharing of attention and a simultaneous response might be linked to striatal DA depletion and disruption of premotor circuits (Duchesne et al 2002). Deficits in tasks involving concurrent cognitive processing have previously been observed in “off” state and de novo PD patients (Malapani et al 1994), further indicating that adequate levels of DA transmission is necessary for simultaneous processing or attention sharing demands. Although these findings of cognitive deficits cannot be attributed to caudate nucleus DA dysfunction alone, these DAT findings do suggest that striatal DA deficiency might contribute to prefrontal cognitive deficits in PD by disrupting fronto–striato–thalamic circuitry loops.

**DAT Studies in Healthy Aging.** A number of molecular imaging studies have demonstrated age-related decreases in DAT density in healthy subjects. These age-related decreases in DAT binding have shown to be a significant mediator of cognitive changes in memory and executive functioning and cognitive performance in general (see Table 1). For instance, Mozley et al (2001) reported age-related reductions of DAT in the putamen and caudate nucleus together with age-related impairments in verbal memory/learning performance. Furthermore, in women but not men, higher striatal DAT binding was associated with better Stroop performance. In a recent study, Erixon-Lindroth et al (2005) further corroborated these age-related reductions in DAT density and associations with age-related decline in episodic memory and executive functioning. Importantly however, the influence of age on cognitive performance was essentially eliminated after statistical control of DAT availability. Furthermore, the finding of a positive association between DAT and crystallized intelligence, which did not vary with age, indicates a general role of DAT availability in cognitive function, over and above age.

**Overview of Presynaptic Dopaminergic Markers and Cognitive Function.** Studies that have examined presynaptic DA markers in relation to cognition suggest an important role of DA synthesis and uptake in modulation of human cognitive processes. Delineating the specific processes regulated by DA and the precise regions mediating these processes from the aforementioned reports, however, is difficult, owing to the different populations examined and variations within populations. For instance, studies examining PD patients varied in disease severity and duration, degree of depression, whether patients were demented, and the patient’s medication status (i.e., on, off, or de novo). Furthermore, not all studies adequately controlled for the effect of motor symptoms on task performance. Differences in the types of cognitive tests used also add significant variation, rendering comparisons across studies difficult. Despite these variations, it seems that in PD and healthy aging, changes in striatal and cortical DA transmission is associated with memory, learning, and a range of prefrontal-type cognitive functions such as response inhibition, attentional set-shifting, working memory, and sustained attention. Impairments in episodic memory observed in PD might also be related to executive dysfunction (Higgins et al 2003). Such associations between presynaptic DA and frontal-type cognition fits within the existing literature that shows such executive processes to be altered by DAergic challenges in healthy subjects (Harrison et al 2004; Mehta et al 1999; Roesch-Ely et al 2005) and impairment of certain executive processes after L-Dopa withdrawal in PD patients (Lange et al 1992). Associations in these molecular imaging studies would be strengthened by manipulating DA tone to produce changes in cognitive performance.

Owing to the reciprocal connections between the basal ganglia, frontal cortex, limbic, and brainstem structures, it is difficult to define the specific roles of the striatum and cortex in information processing. Consequently, it is commonly viewed in terms of frontostriatal or fronto–striato–thalamic circuitry functions or circuitry, and disruption in any one component of this circuitry can lead to functional alterations in other parts. Thus, striatal, and in particular caudatal, presynaptic DA deficiency in PD and healthy aging likely impairs frontostriatal functioning through disruption of the fronto–striato–thalamic circuitry loops, as has been suggested from functional imaging studies in PD (Owen et al 1998). Although the DAT studies generally did not support the segregation of the putamen and caudate nucleus into respective motor and cognitive functions, the relatively poor spatial resolution of SPECT (which was commonly used in the DAT studies) compared with PET (which was only used in the [18F]FDOPA studies) might have contributed to the lack of this dissociation. Therefore, fronto–striato–thalamic circuitry disruption might occur at the level of the caudate nucleus rather than...
putamen. Alterations of presynaptic DA in cortical regions such as the anterior cingulate and frontal cortex was also associated with working memory, response inhibition, and attentional deficits in PD patients, the mechanisms which might be a function of disease stage. In early PD, nigrostriatal DA depletion might cause a compensatory upregulation of DA brought about by increased AADC activity in the mesocortical system, which might differentially affect some frontal cognitive functions (Bruck et al 2005). In advanced PD, however, DA deficiency extends to the frontal cortex, possibly due to progressive denervation of the mesocortical DA pathway, and might directly interfere with PFC functions such as working memory (Rinne et al 2000). Therefore, both nigrostriatal and mesocortical DA alterations might contribute to certain cognitive changes in PD and healthy subjects, either directly, or indirectly through disrupting fronto–striatal neuronal loops.

**DA Receptors and Cognitive Function**

**Dopamine D₂ Receptors.** Experimental and blood flow studies in animals and humans have demonstrated some relationships between D₂ receptors, presumably acting within the striatum, and cognitive processes, particularly in switching behavior and aspects of working memory and planning (Arnsten et al 1995; Mehta et al 1999, 2003). Molecular imaging studies have recently explored the influence of striatal D₂ receptor changes on cognition in aging and certain neurological and psychiatric disorders (Table 2).

Like DA transporters, normal age-related loss of DA receptors in both striatal (Ichise et al 1998) and extrastriatal (Kaasinen et al 2000) areas has been established. Consistent with the DAT findings, this loss of striatal D₂ receptors (indicated by decreased [¹¹C]raclopride binding) in healthy aging is also associated with certain cognitive deficits. Similarly, the deficits have been observed in episodic memory and executive function but also extend to perceptual speed processes (Backman et al 2000; Reeves et al 2005; Volkow et al 1998). Mirroring the findings obtained with DAT, D₂ receptor loss strongly accounted for cognitive impairment irrespective of chronological age, although a paradoxical relationship has been observed between D₁ binding and spatial working memory (Reeves et al 2005). In general, however, these results provide further support that D₂ergic neurotransmission contributes to age-related cognitive decline and cognitive variation in general.

Decreases in striatal D₂ receptors might contribute to cognitive impairments in certain neurological and psychiatric disorders. For instance, D₂ receptor reductions, and to a lesser extent D₁ reductions, in the caudate nucleus and putamen were associated with impairments in a number of executive function tasks measuring planning, memory, response inhibition, and sequencing processes in asymptomatic individuals with Huntington’s disease (Lawrence et al 1998). Likewise, in schizophrenic patients, decreased striatal D₂ receptor density was associated with poorer performance on an attentional task involving discriminating targets from non-targets but only when the effects of time were considered (Yang et al 2004), suggesting that tasks that involve time constraints or require optimal timing might be related to striatal DA activity. Not all patients were neuroleptic-free, however, and although dosage of neuroleptic was controlled for in the analysis, the precise nature of the relationship between striatal D₂ receptor changes and the cognitive task cannot be determined. Nevertheless, the notion of optimal timing of processing is worthwhile to explore in light of research relating timing mechanisms to basal ganglia function. It has been suggested that temporal processing of brief intervals is reliant on D₂ergic activity in the basal ganglia (Rammsayer 1999). Parkinson’s disease patients, who are characterized by severe nigrostriatal denervation, have also been reported to show temporal processing deficits (Harrington et al 1998). Consistent with this notion, several of the measures that have shown associations with D₂ receptor binding, as well as presynaptic DA markers, in the striatum involve time pressure or are dependent on optimal processing within short durations (e.g., processing speed tasks and attentional tasks such as the Stroop). In addition, many of the tasks associated with striatal DA also rely on suppressing attention to competing information or on efficient sequential ordering

**Table 2.** Molecular Imaging Studies Showing Associations Between Postsynaptic Dopamine Receptors and Cognition in Healthy Subjects and Patient Populations

<table>
<thead>
<tr>
<th>Study</th>
<th>Target</th>
<th>Tracer</th>
<th>Population</th>
<th>Cognitive Task(s)</th>
<th>Brain Region</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lawrence et al 1998</td>
<td>D2</td>
<td>[¹¹C]raclopride</td>
<td>HD</td>
<td>Verbal fluency, pattern recognition, spatial span, modified TOL, sequence generation</td>
<td>Caudate and putamen</td>
<td>Decreased binding associated with worse performance</td>
</tr>
<tr>
<td>Volkow et al 1998</td>
<td>D2</td>
<td>[¹¹C]raclopride</td>
<td>Healthy</td>
<td>WCST, Stroop, RSPM, SDMT</td>
<td>Caudate</td>
<td>Decreased binding associated with worse performance</td>
</tr>
<tr>
<td>Backman et al 2000</td>
<td>D2</td>
<td>[¹¹C]raclopride</td>
<td>Healthy</td>
<td>Dots, trail making test A, word and face recognition</td>
<td>Caudate and putamen</td>
<td>Decreased binding associated with worse performance</td>
</tr>
<tr>
<td>Yang et al 2004</td>
<td>D2</td>
<td>[¹²³I]IBZM</td>
<td>Schizophrenia</td>
<td>Tai-Ta attention test</td>
<td>Striatum</td>
<td>Decreased binding associated with worse performance</td>
</tr>
<tr>
<td>Reeves et al 2005</td>
<td>D2</td>
<td>[¹¹C]raclopride</td>
<td>Healthy women</td>
<td>CANTAB TOL and SWM</td>
<td>Caudate and putamen</td>
<td>Decreased binding associated with worse TOL and better SWM</td>
</tr>
<tr>
<td>Okubo et al 1997</td>
<td>D1</td>
<td>[¹¹C]CISH-23390</td>
<td>Schizophrenia</td>
<td>WCST</td>
<td>Prefrontal cortex</td>
<td>Decreased binding associated with worse performance</td>
</tr>
<tr>
<td>Lawrence et al 1998</td>
<td>D1</td>
<td>[¹¹C]CISH-23390</td>
<td>HD</td>
<td>Spatial span, sequence generation</td>
<td>Caudate and putamen</td>
<td>Decreased binding associated with worse performance</td>
</tr>
<tr>
<td>Abi-Dargham et al 2002</td>
<td>D1</td>
<td>[¹¹C]JNNC-112</td>
<td>Schizophrenia</td>
<td>N-back task</td>
<td>DLPFC</td>
<td>Increased binding associated with worse performance</td>
</tr>
</tbody>
</table>

HD, Huntington’s disease; TOL, Tower of London; WCST, Wisconsin Card Sort Test; RSPM, Raven’s Standard Progressive Matrices; SDMT, Symbol Digit Modalities Test; CANTAB, Cambridge Neuropsychological Test Automated Battery; SWM, spatial working memory; DLPFC, dorsolateral prefrontal cortex.

www.sobp.org/journal
and generation of responses, fitting with current proposals that the basal ganglia acts to inhibit competing actions and is involved in optimal organization of information (Casey et al. 2002; Dubois and Pillon 1995; Graybiel 1998; Mink 1996). Associations have also been observed, however, between striatal DA changes and tests of episodic, recognition, and working memory, which do not fit within this framework of basal ganglia function. Therefore, although striatal DA changes are likely to directly affect some fundamental aspects relating to attentional control and temporal processing, such delineations cannot be clearly determined with these studies. Similar to the preceding section, striatal D2 receptor alterations might contribute to cognitive changes via demodulation of the fronto–striato–thalamic circuitry, and performance on these tasks should be largely viewed within the context of this circuitry.

**Dopamine D1 Receptors.** Over the past decade, converging work in monkeys, neuronal recordings, and computational models have indicated the involvement of D1 receptors in modulating and stabilizing prefrontal cortical networks and functions. Local application of specific D1 receptor agonists and antagonists into the PFC of monkeys has mediated spatial working memory performance in a dose-dependent manner (Cai and Arnsten 1997; Sawaguchi and Goldman-Rakic 1991, 1994) and via modulation of pyramidal neurons, or “memory fields” of the prefrontal cortex (Williams and Goldman-Rakic 1995). In humans, administration of a mixed D1/D2 agonist, pergolide, has improved working memory performance (Muller et al. 1998), although investigation of the role of D1 receptors in human cognitive processes has been limited, owing to the lack of selective D1 compounds for human use.

Two PET studies have observed a relationship between D1 receptor density in the PFC and working memory performance in neuroleptic-naïve or -free schizophrenic patients (see Table 2). Reports have been conflicting, however, with both decreases (Okubo et al. 1997) and increases (Abi-Dargham et al. 2002) in PFC D1 receptor binding being associated with impairments in working memory, assessed by the Wisconsin Card Sort Test and n-back task. The main difference between these PET studies is that the former study, showing decreased D1 receptors, used the D1 radioligand [11C]SCH23390, whereas the latter study, reporting increased ligand binding, used the radioligand [11C]NCC-112. The a priori hypothesis of the investigators of these two studies presumably was that patients would have decreased D1 receptor levels, on the basis of the DA hypothesis of schizophrenia that postulates that positive symptoms are associated with increased “DA function” in subcortical regions, whereas cognitive impairment is caused by decreased “DA function” in prefrontal cortex (Davis et al. 1991). The definition of “DA function,” however, is so vague that it can imply even contradictory changes in individual components of the system. For example, Okubo et al. (1997) claim that their finding of decreased D1 receptor binding is consistent with overall decreased “DA function” in prefrontal cortex. With apparently equal face validity, Abi-Dargham et al. (2002) postulate that the increased D1 receptor binding represents denervation upregulation. Because of the relative paucity of studies in this area, neither of these two interpretations can be discounted.

The reasons for these contradictory results are unknown but worthy of further investigation, especially in light of the robustness of studies of prefrontal DA depletion and D1 receptor function in nonhuman primates. The two patient populations seemed fairly similar, at least within our current ability to distinguish subgroups of patients with schizophrenia. An intriguing possibility is that the two PET radioligands (SCH23390 and NNC-112) respond differentially to DA depletion in terms of internalization and availability of binding to the ligand (Guo et al. 2003). The effects of internalization on PET ligand binding is clearly worthy of further investigation for the D1 receptor and the entire class of G-protein coupled receptors, which show such effects on agonist binding.

**Dopamine Release and Cognitive Function**

Neuroreceptor imaging with PET can not only be used to measure the specific molecular target but also to estimate synaptic concentration of endogenous neurotransmitters. The basis of this approach is that radioligands compete with the endogenous neurotransmitter for binding to receptors. Thus, higher synaptic neurotransmitter concentrations are associated with lower radioligand binding and vice versa.

Since the late 1980s, this technique has been used to estimate the concentration of endogenous synaptic dopamine by using D2 receptor radioligands such as [123I]IBZM with PET and [123I]IBZM with SPECT. Two types of release can be estimated: 1) phasic or stimulant-induced DA release, by comparing D2 radioligand binding at baseline with challenge conditions that increase synaptic DA levels, such as d-amphetamine; and 2) tonic DA release, by comparing baseline radioligand binding with transient DA depleted conditions, such as that induced by the tyrosine hydroxylase inhibitor alpha-methyl-para-tyrosine (AMPT). Such increases and decreases in synaptic DA levels have induced decreases and increases, respectively, in D2 binding potential (for review, see Laruelle 2000).

Although alterations in striatal DA release have been observed in schizophrenia (Breier et al. 1997; Laruelle et al. 1996), PD (Piccini et al. 2003), and chronic cocaine abuse (Volkow et al. 1997) using pharmacological challenges, such alterations have not been related to cognitive performance. However, preliminary studies suggest that behavioral challenges might also be used to induce DA release in healthy subjects, therefore reflecting task-related regional DA release. In a landmark study, Koepf et al. (1998) showed decreased [123I]Iodo-claclone binding in the dorsal and ventral striatum during performance of a video game, which involved learning to navigate a tank through a battlefield for a financial incentive, compared with baseline. This associated striatal DA release with sensorimotor coordination, learning actions that predict reward, and anticipating rewards, complementing previous findings seen in animals. Recently, two studies have measured extrastriatal DA neuromodulation with cognitive tasks in healthy volunteers. Reduced [11C]FLB457 binding was observed in the ventrolateral frontal cortex, medial temporal cortex, and ventral anterior cingulate during a working memory and sustained attention task (Aalto et al. 2005), whereas decreased [18F]Fla-pro binding in the thalamus was detected during a spatial attention task and, importantly, highly correlated with task performance (Christian et al., in press). These studies provide further evidence that extrastriatal DA transmission is critical for working memory and attentional processes.

Changes in tonic or baseline DA levels have also been estimated by increased [123I]Iodo-claclone binding after AMPT in the striatum (Laruelle et al. 1997; Verhoeff et al. 2001) and most recently after depletion of tyrosine and phenylalanine (TPD) (Mehta et al. 2005) in healthy subjects. Although increased [13C]Iodo-claclone binding (indicating reduced tonic DA) was associated with worse sustained attention (Verhoeff et al. 2001), this was not replicated in a subsequent study (Verhoeff et al. 2003).
and might have been confounded by detrimental effects of AMPT on mood and arousal; however, with TPD, participants who became impaired in spatial working memory and planning accuracy were more likely to show greater reductions of tonic DA in the striatum (Mehta et al. 2005). The latter findings suggest that changes in striatal DA levels can modulate certain cognitive processes and that the level of cognitive impairment is dependent on the level of DA depletion.

Positron emission tomography studies that have measured DA release and cognitive performance are summarized in Table 3; however, interpreting such relationships requires examination of the meaning of “DA function.” As previously discussed, researchers often seek simple understandings of the overall role of “DA function.” Is it increased or decreased? In fact, “DA function” has many components (pre-, intra-, and postsynaptic as well as post-receptor signal transduction); the answers will be more complex than a simple increase or decrease. The studies described here of intrasynaptic DA levels measured by D2 radioligands have been exciting, in part because they purport to isolate one component of DA function. More specifically, this neuroimaging measurement of DA turnover reflects the net effect of DA release and reuptake—as well as the amount of transmitter that diffuses to the D2 receptor, whether located within or adjacent to the synapse. However exciting these measurements might seem, the results should be interpreted with great caution for both technical and theoretical reasons. First, the studies are intended to measure changes in intrasynaptic DA, the D2 receptors themselves are almost certainly altered by the pharmacological challenge. Like most G-protein coupled receptors, agonist binding to the D2 receptor causes uncoupling, internalization, and possibly also dimerization. In large part, we do not know the effects of this receptor trafficking on the imaging measurements, including whether the ligand binds to the internalized receptor and whether specific agents differ in this ability (but see Sun et al. 2003). In the absence of a specific transport mechanism, successful radioligands must have adequate lipophilicity to diffuse across the blood brain barrier—thus, they might have access to the internalized receptor; however, its structure would have changed after uncoupling to the G-protein complex so that it might not bind with the same affinity. Recently, two D2 agonist radioligand analogs of apomorphine have been described (Finnema et al. 2005; Hwang et al. 2000) and might be useful to assess aspects of this problem. In vitro studies have consistently shown that antagonists such as raclopride bind with equal affinity to both the G-protein coupled and uncoupled forms of the receptor. In contrast, agonists (like apomorphine or DA itself) bind with higher affinity to the coupled than the uncoupled forms, which are then called the high- and low-affinity states, respectively. As expected, amphetamine caused a greater displacement of the D2 agonist radioligand, [11C]NPA, than [11C]raclopride binding in monkey striatum, whereas concurrent (1997) found that IV amphetamine caused a 21% decrease of extracellular DA with microdialysis. For example, Breier et al. 

### Table 3.

<table>
<thead>
<tr>
<th>Study</th>
<th>Target</th>
<th>Tracer</th>
<th>Challenge</th>
<th>Cognitive Task(s)</th>
<th>Brain Region</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verhoeff et al 2001</td>
<td>D2</td>
<td>[11C]raclopride</td>
<td>AMPT</td>
<td>Connors continuous performance test</td>
<td>Neostriatum</td>
<td>Increase in binding correlated with worse performance</td>
</tr>
<tr>
<td>Mehta et al 2005</td>
<td>D2</td>
<td>[11C]raclopride</td>
<td>TPD</td>
<td>Modified delayed response task</td>
<td>Dorsal and ventral striatum</td>
<td>Increase in binding correlated with worse performance</td>
</tr>
<tr>
<td>Koepp et al 1998</td>
<td>D2</td>
<td>[11C]raclopride</td>
<td>Motor/cognitive</td>
<td>Modified delayed response task</td>
<td>Striatum (especially ventral)</td>
<td>Decreased binding during task performance compared with baseline</td>
</tr>
<tr>
<td>Aalto et al 2005</td>
<td>D2</td>
<td>[11C]FLB457</td>
<td>Cognitive</td>
<td>N-back task (0-back)</td>
<td>Left ventral AC</td>
<td>Decreased binding during task performance compared with baseline condition</td>
</tr>
<tr>
<td>Christian et al, in press</td>
<td>D2</td>
<td>[18F]fallypride</td>
<td>Cognitive</td>
<td>Spatial attention task</td>
<td>Thalamus</td>
<td>Decreased binding during task performance correlated with better performance</td>
</tr>
</tbody>
</table>

cloride (Narendran et al 2004), because it preferentially labels the form of the receptor with greater affinity for DA. Thus, D_2 agonist radioligands (in conjunction with the standard antagonist radioligands) might be useful to examine the effects of drugs on postsynaptic receptors because they are thought to selectively label the G-protein coupled form of the receptor.

**Summary**

In recent years, molecular imaging with PET and SPECT has provided a new approach to examining DA and its role in a variety of cognitive processes, particularly those subserved by the fronto–striato–thalamic circuitry. In light of the reviewed molecular imaging studies, it seems that a range of cognitive tasks are associated with changes in pre- and postsynaptic components of the DA system within the fronto–striato–thalamic circuitry. The fact that correlations between DA indices and cognitive measures were demonstrated across different diseases such as PD, schizophrenia, and Huntington’s disease as well as healthy aging provides greater strength to these relationships; however, delineating regionally specific components of the DA system critical for specific cognitive processes is difficult for several reasons. First, the striatum is a key focus in DA/cognition relationships partly because it is a large, high-density structure that is easily visualized and quantified. The development of more DA radioligands with high selectivity, specificity, and signal-to-noise activity that can be imaged in extrastriatal regions is therefore needed. Second, it is difficult to refer to specific functions of brain structures, particularly the striatum, in isolation of frontostriatal circuits. As such, associations between DA markers and cognitive measures need to be considered within the context of the fronto–striato–thalamic loops. Third, the cognitive tasks used in the reviewed studies are complex, consisting of multiple components and presumably engaging multiple regions. Nevertheless, consistent relationships were observed between DA indices (particularly D_1 receptors), in cortical areas like the frontal cortex, and working memory and sustained attention performance, suggesting that cortical DA transmission is critical for maintaining and representing on-going responses, as has been proposed by others (Bilder et al 2004; Casey et al 2002). In contrast, the observed relationships between striatal DA alterations (particularly D_2 receptors) and a variety of cognitive tasks might be related to motor performance as well as inhibition of competing responses, temporal processing, and sequential organization of behavior (see preceding sections). Striatal DA alterations, however, might not directly interfere with specific cognitive performance but might do so indirectly by disrupting the fronto–striato–thalamic circuitry.

It must be noted that, owing to the slow kinetics of DA radioligands, most of the PET and SPECT studies of DA and cognition are correlational in nature and do not measure cognition at the time of the imaging measurement. As such, no causative roles can be implied. Molecular imaging (as opposed to functional imaging) cannot temporally track different components of the cognitive task at hand, which might be differentially regulated by DA function. Future studies might therefore benefit from assessing simple cognitive processes rather than complex tasks and should attempt to control for the effects of novelty, task difficulty, and sensory and motor function on cognitive performance and DA activation. Lastly, imaging the influence of genes (such as the catechol-O-methyl transferase gene variant, an enzyme critical for PFC DA flux) on DA and cognitive function (see Mattay and Goldberg 2004) will no doubt be a major focus in future molecular imaging studies.


Mehta MA, Gumaste D, Montgomery AJ, McTavish SF, Grasby PM (2005): The effects of acute tyrosine and phenylalanine depletion on spatial working memory and planning in healthy volunteers are predicted by changes in striatal dopamine levels. Psychopharmacology (Berl) 180:654–663.


www.sobp.org/journal


www.sobp.org/journal
APPENDIX 2: Reprint of Cropley et al. 2008 (Synapse)
Small Effect of Dopamine Release and No Effect of Dopamine Depletion on [18F]Fallypride Binding in Healthy Humans

VANESSA L. CROPLEY,1,2 ROBERT B. INNIS,1 PRADEEP J. NATHAN,3 AMIRA K. BROWN,1 JANET L. SANGARE,1 ALICJA LERNER,1 YONG HOON RYU,1,4 KELLY E. SPRAGUE,1 VICTOR W. PIKE,1 AND MASAHIRO FUJITA1*

1Molecular Imaging Branch, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland
2Brain Sciences Institute, Swinburne University of Technology, Hawthorn, Victoria, Australia
3School of Psychology, Psychiatry and Psychological Medicine, Monash University, Clayton, Victoria, Australia
4Department of Nuclear Medicine, Yonsei University Medical College, Seoul, South Korea

KEY WORDS D2 radioligand; d-amphetamine; α-methyl-para-tyrosine; phasic; tonic; PET

ABSTRACT Molecular imaging has been used to estimate both drug-induced and tonic dopamine release in the striatum and most recently extrastriatal areas of healthy humans. However, to date, studies of drug-induced and tonic dopamine release have not been performed in the same subjects. This study performed positron emission tomography (PET) with [18F]fallypride in healthy subjects to assess (1) the reproducibility of [18F]fallypride and (2) both d-amphetamine-induced and α-methyl-p-tyrosine (AMPT)-induced changes in dopamine release on [18F]fallypride binding in striatal and extrastriatal areas. Subjects underwent [18F]fallypride PET studies at baseline and following oral d-amphetamine administration (0.5 mg/kg) and oral AMPT administration (3 g/70 kg/day over 44 h). Binding potential (BP) (BPND) of [18F]fallypride was calculated in striatal and extrastriatal areas using a reference region method. Percent change in regional BPND was computed and correlated with change in cognition and mood. Test–retest variability of [18F]fallypride was low in both striatal and extrastriatal regions. D-Amphetamine significantly decreased BPND by 8–14% in striatal subdivisions, caudate, putamen, substantia nigra, medial orbitofrontal cortex, and medial temporal cortex. Correlation between change in BPND and verbal fluency was seen in the thalamus and substantia nigra. In contrast, depletion of endogenous dopamine with AMPT did not effect [18F]fallypride BPND in both striatum and extrastriatal regions. These findings indicate that [18F]fallypride is useful for measuring amphetamine-induced dopamine release, but may be unreliable for estimating tonic dopamine levels, in striatum and extrastriatal regions of healthy humans. Synapse 62:399–408, 2008. Published 2008 Wiley-Liss, Inc.

INTRODUCTION Molecular imaging with single photon emission computed tomography (SPECT) or positron emission tomography (PET) can be used not only to measure D2 receptor density, but also, under appropriate conditions, to estimate synaptic concentration of endogenous dopamine. This approach is based on the competition between certain radioligands and endogenous dopamine for D2 receptor binding, according to pharmacological theories defined by an occupancy model (for review see Laruelle, 2000). As such, changing the concentration of dopamine will affect the number of available D2 receptors, with increases of dopamine reducing D2 receptor availability (i.e., specific binding) and vice versa.
Over the past decade, SPECT or PET D₂ receptor measurement coupled with pharmacological interventions either to increase synaptic dopamine levels with a stimulant acutely or deplete dopamine levels (tonic release) rapidly, have been used to examine synaptic dopamine transmission in human brain (for review see Laruelle, 2000). However, the majority of these studies have been confined to the striatum, since the striatum is a relatively large region with an abundance of D₂ receptors (Kessler et al., 1993). Stimulating dopamine release with either intravenous or oral doses (0.3 mg/kg or 30 mg) of d-amphetamine (herein referred to as amphetamine) in healthy subjects has consistently decreased striatal binding of [¹⁸F]fallypride (Boileau et al., 2006; Cardenas et al., 2004; Dreven et al., 2001; Leyton et al., 2002; Martinez et al., 2003) and [¹²³I]IBZM (Kegeles et al., 1999; Laruelle et al., 1995) by 7–18%. In contrast, depletion of cerebral dopamine with α-methyl-p-tyrosine (AMPT) (Engelman et al., 1968), a competitive inhibitor of tyrosine hydroxylase, increased [¹⁴C]raclopride and [¹²³I]IBZM binding in striatum by 9–28% (Abi-Dargham et al., 2000; Laruelle et al., 1997; Verhoeff et al., 2001, 2002). Increase in radioligand binding was suggested to be due to removal of endogenous dopamine, subsequently unmasking D₂ receptors previously occupied by it and thus providing a measure of baseline or tonic dopamine release (Laruelle et al., 1997).

Although assessment of striatal dopamine release is important for increasing our understanding of a number of psychiatric and neurological disorders, a number of studies indicate the involvement of extrastriatal dopamine transmission in schizophrenia, addiction, neuroleptic drug interactions, and various cognitive processes (Arnsten, 1998; Laviolette and Grace, 2006; Lidow et al., 1998). Recent focus has been directed to developing high-affinity SPECT and PET radioligands that enable quantification of low-density extrastriatal dopamine receptors. [¹⁸F]Fallypride is a high-affinity D₂/D₃ radioligand (Kᵩ = ~0.2 nM) (Slifstein et al., 2004a) which, with its high specific-to-nondisplaceable binding, is capable of measuring D₂-type receptors in striatal, as well as extrastriatal regions such as thalamus, temporal cortex, substantia nigra, and limbic areas (Mukherjee et al., 2002). [¹⁴F]Fallypride is sensitive to changes in extracellular levels of endogenous dopamine, both in the striatum and extrastriatal regions. In nonhuman primates, a 14–49% displacement of [¹⁸F]Fallypride was reported in the striatum, thalamus, amygdala, hippocampus and pituitary, following 0.6–1 mg/kg intravenous dose of amphetamine (Mukherjee et al., 1997, 2005; Slifstein et al., 2004b). Recently, amphetamine-induced displacement of [¹⁸F]fallypride binding was demonstrated in both striatal and extrastriatal regions in healthy volunteers following an oral dose of 0.43 mg/kg amphetamine (Riccardi et al., 2006).

Displacement of [¹⁸F]fallypride was greatest in striatal subdivisions (6–11%) and substantia nigra (7%), with lesser displacement seen in amygdala, temporal cortex, and thalamus (3–4%). In addition, depletion of dopamine with AMPT was recently reported to increase [¹⁸F]fallypride binding by 9–13% in striatal subdivisions and substantia nigra in a small group of healthy volunteers (Riccardi et al., 2008). To date, no study has examined the effects of drug-induced and tonic dopamine release on striatal and extrastriatal D₂ receptors within the same subjects. Although drug-induced dopamine release may be different from physiological phasic release, given the close relationship between these two modes of dopamine transmission, with tonic release modulating phasic release (Grace, 1991), we wanted to establish the effect of within-subject changes in both amphetamine-induced and tonic dopamine on [¹⁸F]fallypride binding. Such within-subject examination may aid in understanding the interactions between phasic and tonic dopamine release.

The current study performed [¹⁸F]fallypride PET scans at baseline and following oral amphetamine and AMPT administration in healthy human volunteers. The purpose of the study was to; (1) assess the reproducibility of measuring [¹⁸F]fallypride binding (i.e., test–retest reliability), (2) for the first time, examine within the same subjects, the effects of amphetamine-induced dopamine release and AMPT-induced tonic dopamine depletion on [¹⁸F]fallypride binding in both striatum and extrastriatal areas, and (3) examine the within-subject relationship between amphetamine-induced and tonic dopamine release as measured by [¹⁸F]fallypride binding in both striatum and extrastriatal areas. On the basis of previous studies and the hypothesis put forth by Grace (1991), we hypothesize that amphetamine and AMPT pretreatment will decrease and increase [¹⁸F]fallypride binding, respectively, and that subjects with greater amphetamine-induced release will have smaller tonic release.

**METHODS**

**Study population**

Fourteen healthy volunteers (11 male, 3 female; mean age ± SD, 29 ± 8 years; range, 20–43 years) participated in the study. All subjects were right-hand dominant and none were smokers. Subjects were free of current medical, psychiatric, and neurological illness based on history, a physical exam, routine laboratory tests (including a complete blood count, chemistries, thyroid function test, serum electrolytes, liver and kidney function, urinalysis, urine drug screen and HIV and Hepatitis B tests), and electrocardiogram. Exclusion criteria included evidence of current psychiatric or neurological condition, medi-
TABLE I. Participant demographics and clinical measures

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Education (years)</th>
<th>Amphetamine plasma level at 3 h after administration (ng/ml)</th>
<th>AMPT plasma level (µg/ml)</th>
<th>[^{18}F]fallypride injected activity (MBq)</th>
<th>[^{18}F]fallypride specific activity (GBq/µmol)</th>
<th>Interval between scan 1 and 2 (test-retest) (days)</th>
<th>Interval between scan 2 (retest) and 3 (amphetamine) (days)</th>
<th>Interval between scan 3 (amphetamine) and 4 (AMPT) (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>29 ± 8</td>
<td>16 ± 2</td>
<td>62 ± 19.2</td>
<td>20 ± 4.3</td>
<td>187 ± 11</td>
<td>70 ± 37</td>
<td>15</td>
<td>31</td>
<td>42</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation.

*Value is median.

cally significant biochemical or hematological abnormality, elevated blood pressure (>140/90), history of myocardial infarction or angina pectoris, positive urine drug screen, history of substance abuse or dependence within 6 months, body weight greater than 93 kg and pregnancy and lactation. The Radiation Safety Committee of the National Institutes of Health and the Institutional Review Board of the National Institute of Mental Health approved the study. All subjects gave written informed consent.

**Radiopharmaceutical preparation**

\[^{18}F\]Fallypride was synthesized via nucleophilic substitution of tosyl-fallypride (ABX GmbH, Radeberg, Germany) with cyclotron-produced \[^{18}F\]fluoride ion (Mukherjee et al., 1995). Preparations were conducted according to our Investigational New Drug Application no. 70,046, submitted to the US Food and Drug Administration and a copy of which is available at: http://kidb.bioc.cwru.edu/snidd/.

**Scanning protocol**

PET scans were acquired in three-dimensional mode using a GE Advance tomograph (GE Medical Systems, WI) and were reconstructed with the filtered-back projection algorithm which resulted in a final image resolution of 7.5 mm full width half maximum. Following the initial screening visit, subjects underwent four \[^{18}F\]fallypride PET scans on four separate days in the following order – two baseline scans (test-retest) (scan 1 and 2), a scan with amphetamine administration (scan 3), and a scan with AMPT administration (scan 4). The median interval between baseline test–retest scans was 2 weeks, while there was about a 1 month interval between scan 2 (retest) and scan 3 (amphetamine) and 6 weeks between scan 3 (amphetamine) and scan 4 (AMPT) (Table I). All female subjects underwent a pregnancy test within 24 h before each PET scan. In all PET studies, an 8-min transmission scan using a \(^{68}\)Ge rotating pin source was performed for attenuation correction. Dynamic emission scans were acquired following an intravenous bolus injection of 187 ± 11 MBq \[^{18}F\]fallypride for 3 h (specific activity = 70 ± 37 GBq/µmol; mass dose/body weight = 0.017 ± 0.008 µg/kg). The initial image acquisition coincided with \[^{18}F\]fallypride injection and was obtained continuously for 60 min (6 × 30 s, 3 × 1 min, 2 × 2 min, 10 × 5 min). Following this initial acquisition, two 1-h images (12 × 5 min) were acquired until about 5 h after the bolus injection. Subjects were removed from the camera for about 60 min between image acquisitions. To ensure metabolism of the radioligand was consistent among scans, subjects did not eat from 2 h before \[^{18}F\]fallypride administration to the completion of the scan, and half normal saline was intravenously infused at a rate of 83 ml/h starting 1 h before \[^{18}F\]fallypride injection. Subjects also consumed at least 1 l of water before and during intermissions of the PET scan. To minimize potential changes in dopamine levels, subjects were asked to refrain from consuming caffeine-containing drinks after midnight before each PET scan.

On the day of PET scan 3, a single oral dose of 0.5 mg/kg amphetamine (Dexedrine\textsuperscript{®}) was administered 3 h before injection of \[^{18}F\]fallypride with vital sign monitoring. Scanning was performed 3 h following amphetamine administration because there is sustained radioligand displacement following oral amphetamine (Cardenas et al., 2004). We used a slightly higher dose (0.5 mg/kg) than in previous studies (0.3–0.43 mg/kg) to induce greater dopamine release and to more easily detect changes in \[^{18}F\]fallypride binding. Plasma amphetamine levels were measured at about 3 and 4.5 h following amphetamine administration to measure amphetamine levels during the PET scanning period. Plasma samples were analyzed for amphetamine using an isotope dilution procedure. The method was a minor modification of the procedure of Xie et al. (2004). The modification for plasma involved initial protein precipitation with sulfosalicylic acid. The clear supernatant was then processed as described for ultrafiltrates in Xie et al. (2004). The standard curve was linear (r = 0.999) in the range tested (1–500 ng/ml). The limit of detection was 1 ng/ml with intra and inter coefficients of variation of <5% and <7% respectively.

On the day of PET scan 4, subjects were administered 3 g/70 kg body weight AMPT (Demser\textsuperscript{®}) p.o. per day over 44 h (10 doses in total) (Fujita et al., 2000; Laruelle et al., 1997). Although Laruelle et al. (1997) did not adjust AMPT dose based on body weight, they administered almost the same dose for the same length of time. Subjects with body weight more than 93 kg were excluded from the study to limit AMPT dose to 4 g/day. To prevent crystalluria during AMPT administration, subjects were asked to drink 1–2 l of water each day. In addition, half-normal saline was infused intravenously until 3 h after

*Synapse*
the end of the fourth PET scan. A urinalysis and medical examination was conducted daily to detect crystal formation and assess tolerance to AMPT treatment. In addition, sodium bicarbonate (2 tablets of 650 mg) was given on three consecutive nights to prevent acidification of the urine during the night. To determine levels of AMPT in plasma, blood samples were collected about 90 min before, and 90 min and 3.5 h after \(^{18}\)Ffallypride injection. Plasma levels of AMPT were determined by high-performance liquid chromatography (HPLC) using a modification of a previously reported method for the determination of plasma tryptophan and kynurenine (Hoekstra et al., 2007). In brief, 100 \(\mu\)l plasma samples were deproteinized with 100 \(\mu\)l of 0.7 M perchloric acid after addition of 50 \(\mu\)l of 25% ascorbic acid. Supernates obtained after centrifugation were directly injected on a 15 \(\times\) 0.46 cm Microsorb C18 column eluted with 96% pH 3.7, 1.5% aqueous acetic acid and 4% methanol delivered at a flow rate of 1 ml/min, and the compounds detected fluorometrically (270/320 nm excitation/emission wavelengths). All specimens were analyzed in a single assay and the compounds determined with within-assay coefficients of variation of less than 5%.

Three subjects participated in a pilot study of only one baseline and one amphetamine scan. Of the eleven subjects who participated in the 4 scan protocol (test, retest, amphetamine, and AMPT) six subjects completed the fourth scan with AMPT while two subjects commenced the fourth scan but withdrew before completion, allowing measurement in only extrastriatal areas. Therefore, the total number of subjects was 11 for test–retest, 14 for amphetamine-induced change and 6 and 8 for AMPT-induced change in striatum and extrastriatal areas, respectively.

All subjects received a 1.5 Tesla MRI scan for coregistration and segmentation purposes. Inversion recovery fast gradient recalled-echo (IR-FGRE; TR ~12 ms, TE ~5 ms, voxel size: 0.86 \(\times\) 0.86 \(\times\) 1.2 mm), fast spin echo (FSE) T2-weighted (TR ~3700 ms, TE ~101 ms, flip angle 90\(^\circ\), voxel size: 0.43 \(\times\) 0.43 \(\times\) 5 mm) and fluid attenuated inversion recovery (FLAIR; TR ~10,002 ms, TE ~140 ms, flip angle 90\(^\circ\), voxel size: 0.86 \(\times\) 0.86 \(\times\) 5 mm) images were obtained.

Neuropsychological tests

On the morning of each scan, subjects were administered a neuropsychological battery to examine executive, attentional, processing speed, and frontostriatal processes. On amphetamine days, the commencement of the battery corresponded to 60–90 min post amphetamine administration as peak subjective and behavioral effects of oral amphetamine have shown to occur within 1–2.5 h after administration (Angrist et al., 1987; Asghar et al., 2003). For baseline 1 and amphetamine scans the battery consisted of the Symbol Digit Modality task (speed of processing), spatial span (attention and spatial working memory), Stroop color word task (response inhibition, executive function), Controlled Oral Word Association Test – CFL (verbal fluency, executive function), and the Colors Trail Test (processing speed, visual attention). For baseline 2 and AMPT scans, subjects were administered the Digit Symbol Coding test (speed of processing), Controlled Oral Word Association Test - FAS, Colors Trail Test, Letter-Number Sequencing (working memory) and the Stockings of Cambridge task (planning, frontostriatal function). To minimize practice effects, and because of the unavailability of four alternate versions of the neuropsychological tests, different tests were selected for the scans 1 and 3 vs. scans 2 and 4. Tests were administered in a fixed order with alternate forms available for some tests. Similar processes were assessed for each of the drug challenge days. The testing interval was ~8 weeks between the baseline 1 and amphetamine sessions and ~11 weeks between baseline 2 and AMPT sessions. One male and one female subject did not undergo neuropsychological testing following amphetamine treatment due to a previous amphetamine scan cancellation that occurred in close proximity to their retest scan. Two subjects did not complete the Letter-Number Sequencing test and two subjects were not administered the Stockings of Cambridge task due to time constraints on the testing day. Drug-induced changes in subjective mood were assessed using 10-point visual analog scales for euphoria, restlessness, anxiety, drowsiness and alertness and the Profile of Mood States (POMS; McNair et al., 1971). For amphetamine-induced emotional change, subjects rated these questionnaires pre- and about 70 min post amphetamine administration. For AMPT-induced change, subjects were administered the questionnaires at baseline (on the morning of the retest scan) and following AMPT (at the equivalent point in time on the day of the AMPT scan).

Data analysis

To correct for head movement during the scan, all \(^{18}\)Ffallypride frames of each PET scan were realigned to a standard frame using the FLIRT algorithm (Jenkinson and Smith, 2001) for the initial image acquisition and Statistical Parametric Mapping (SPM2, The Wellcome Department of Cognitive Neurology, London, UK) for the second and third acquisitions. FLIRT was used for the initial image acquisition instead of SPM2 because for the initial set, SPM2 cut the bottom portion of images including the cerebellum. Inversion recovery MRI reoriented to the anterior commissure-posterior commissure (AC-PC) line and PET scans 2, 3, and 4 were each coregistered to Synapse
an average image of initial $[^{18}F]$fallypride frames from PET scan 1 using SPM2. Coregistered serial PET scans and MRI were spatially normalized to the Montreal Neurological Institute stereotaxic space using segmented gray matter images created from IR, FLAIR, and T2 MRI images in SPM2. Regions of interest defined on the caudate nucleus, putamen, thalamus, medial orbitofrontal cortex, anterior cingulate, temporal cortex, medial temporal cortex, substantia nigra, and colliculi were applied to spatially normalized PET images. Medial orbitofrontal cortex, substantia nigra, and colliculi volumes were delineated on parametric images and the location of the substantia nigra and colliculi were individually adjusted without knowing the scan identity. Striatal subdivisions were drawn on reoriented MRI and were defined according to the criteria of Mawlawi et al. (2001) for the ventral striatum, precommissural dorsal caudate, and precommissural dorsal putamen and according to Martinez et al. (2003) for the postcommissural caudate and putamen. Partial volume correction was applied to striatal subdivisions in order to recover lost spatial resolution to these regions. Partial volume correction was performed in PMOD 2.65 (pixel-wise modeling computer software; PMOD Technologies Ltd, Adliswil, Switzerland), using a model based on Giovacchini et al. (2004). Both uncorrected and corrected data from striatal subdivisions underwent region of interest (ROI) analysis.

Regional $[^{18}F]$fallypride BP (BP$_{ND}$) was calculated from 5-h data using the simplified reference tissue model (Lammertsma and Hume, 1996) implemented in PMOD by using cerebellum excluding vermis as the reference region. Test–retest variability and percent change in amphetamine and AMPT-induced BP$_{ND}$ were calculated for each ROI. Test–retest variability was calculated as the absolute difference of baseline scan 1 (test) minus baseline scan 2 (retest) divided by the mean of test–retest and expressed as a percent. Amphetamine or AMPT-induced change in $[^{18}F]$fallypride BP$_{ND}$ was determined as the percent difference in BP$_{ND}$ between the mean baseline (scan 1 and 2) and postamphetamine (scan 3) or post-AMPT (scan 4) conditions: $\Delta$BP$_{ND}$ = (BP$_{ND}$ (drug) - BP$_{ND}$ (mean baseline))/BP$_{ND}$ (mean baseline) $\times$ 100. Changes in cognition from baseline to amphetamine or AMPT conditions were calculated by subtracting baseline values from drug (amphetamine or AMPT) values and dividing by baseline. Drug-induced change in mood was assessed as the difference between pre- (or baseline) and postdrug ratings on each VAS and POMS dimension.

In addition, mean parametric images of changes in $[^{18}F]$fallypride BP$_{ND}$ were calculated (Gunn et al., 1997) and voxel-wise analysis of change images restricted to the entire lateral temporal cortex (i.e., small volume correction) was performed using SPM2 and SnPM, the nonparametric version of SPM. This was done because $[^{18}F]$fallypride showed binding in the entire temporal cortex, and changes in an area in the temporal cortex may have been overlooked by applying regions of interest.

**Statistical analysis**

For 11 subjects who had two baseline scans (test and retest), kinetic analysis was performed for each scan and the average values were used as baseline measurements. To determine the effects of amphetamine or AMPT on $[^{18}F]$fallypride BP$_{ND}$, repeated-measures analysis of variances (ANOVA) with condition (baseline, amphetamine or baseline, AMPT) and region as within-subject factors, were performed using SPSS for Windows (SPSS, 1989–2004, Release 14.0). Greenhouse-Geisser corrections were used for violations of the assumption of sphericity. Separate ANOVAs were performed for striatal subdivisions and other regions (striatum and extrastriatal regions). When appropriate, paired-sample $t$-tests or Wilcoxin signed ranks tests for parametric and nonparametric data respectively, were performed to determine which regions accounted for the significant effects found on the ANOVA. Parametric and nonparametric variables were determined by the Shapiro-Wilk normality test. Right and left sided differences in regional drug-induced displacement of BP$_{ND}$ were examined with paired $t$-tests or Wilcoxin signed rank tests. Relationships between change in BP$_{ND}$ and change in cognition and mood, and between amphetamine and AMPT-induced change in BP$_{ND}$, were performed with Spearman’s rank correlation. Multiple comparisons were controlled for with a false discovery rate correction (Benjamini and Hochberg, 1995).

**RESULTS**

$[^{18}F]$Fallypride uptake and BP$_{ND}$

$[^{18}F]$Fallypride was visualized and quantified in both the striatum and extrastriatal regions such as thalamus, temporal cortex, anterior cingulate, and orbitofrontal cortex (Fig. 1). BP in striatal areas were about 10–30-fold higher than values in extrastriatal areas.

**Test–retest variability**

Test–retest variability of $[^{18}F]$fallypride BP$_{ND}$ was low for most regions, ranging from ~3.8% in the striatum, ~5% in medial and temporal cortex, to 6–8% in thalamus, medial orbitofrontal cortex, and substantia nigra. Intraclass correlation coefficient (ICC) was above 0.90 (range: 0.91–0.98) for these regions (Table II). Two regions showed test–retest variability above 10%; these were the anterior cingulate (test–retest = 21.8%, ICC = 0.54) and colliculi (test–retest = 10.9%, Synapse
ICC = 0.72) (Table II). Given the poor reproducibility and low reliability of BP\textsubscript{ND} in these regions, the anterior cingulate and colliculi were not included in further analysis of amphetamine and AMPT effects. Test–retest variability was excellent in all striatal subdivisions, ranging from 3 to 5.6% and showing ICC above 96% (Table III). Partial volume correction of striatal subdivisions resulted in poorer test–retest variability of BP\textsubscript{ND} (4% to 6%) compared with uncorrected striatal subdivisions (~4%).

**Amphetamine-induced changes**

A repeated-measures ANOVA using condition, region and condition by region as factors showed significant main effects for condition \((F(1,13) = 11, P = 0.006)\), region \((F(6,78) = 335, P < 0.001)\) and a significant condition by region interaction \((F(6,78) = 12.4, P = 0.003)\). The effect of amphetamine on each region was further examined by paired two-tailed \(t\)-tests. These tests, with correction for multiple comparisons using the false discovery rate (Benjamini and Hochberg, 1995), revealed significant amphetamine-induced decreases in \(^{18}\text{F}\)fallypride BP\textsubscript{ND} in, by rank order, the substantia nigra (13.1%), medial orbitofrontal cortex (13%), putamen (12.3%), medial temporal cortex (7.8%), and caudate (7.6%) (Table IV). Lower levels of displacement were seen in the thalamus and temporal cortex (7%) but these did not reach significance. For striatal subdivisions, a repeated-

---

**TABLE II. Test-retest reproducibility of measuring \(^{18}\text{F}\)fallypride binding potential (BP\textsubscript{ND})**

<table>
<thead>
<tr>
<th>Region</th>
<th>Test</th>
<th>Retest</th>
<th>Test–retest variability (%)</th>
<th>ICC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caudate</td>
<td>17.1 ± 1.0</td>
<td>17.4 ± 1.1</td>
<td>3.8 ± 0.7</td>
<td>0.98</td>
</tr>
<tr>
<td>Putamen</td>
<td>19.8 ± 1.3</td>
<td>19.8 ± 1.4</td>
<td>3.7 ± 0.8</td>
<td>0.98</td>
</tr>
<tr>
<td>Thalamus</td>
<td>1.61 ± 0.08</td>
<td>1.68 ± 0.08</td>
<td>6.1 ± 1.3</td>
<td>0.91</td>
</tr>
<tr>
<td>Medial orbitofrontal cortex</td>
<td>0.60 ± 0.05</td>
<td>0.64 ± 0.06</td>
<td>6.7 ± 1.8</td>
<td>0.95</td>
</tr>
<tr>
<td>Anterior cingulate</td>
<td>0.55 ± 0.05</td>
<td>0.59 ± 0.05</td>
<td>21.8 ± 3.8</td>
<td>0.54</td>
</tr>
<tr>
<td>Temporal cortex</td>
<td>0.72 ± 0.07</td>
<td>0.72 ± 0.07</td>
<td>4.8 ± 1.2</td>
<td>0.98</td>
</tr>
<tr>
<td>Medial temporal cortex</td>
<td>0.86 ± 0.06</td>
<td>0.90 ± 0.07</td>
<td>5.1 ± 0.9</td>
<td>0.96</td>
</tr>
<tr>
<td>Substantia nigra</td>
<td>1.17 ± 0.09</td>
<td>1.16 ± 0.10</td>
<td>7.7 ± 2.0</td>
<td>0.95</td>
</tr>
<tr>
<td>Colliculi</td>
<td>1.52 ± 0.06</td>
<td>1.44 ± 0.08</td>
<td>10.9 ± 2.6</td>
<td>0.72</td>
</tr>
</tbody>
</table>

ICC, intraclass correlation coefficient. Data are mean ± SEM.

**TABLE III. Test-retest reproducibility of measuring \(^{18}\text{F}\)fallypride binding potential (BP\textsubscript{ND}) in striatal subdivisions**

<table>
<thead>
<tr>
<th>Region</th>
<th>Test</th>
<th>Retest</th>
<th>Test–retest variability (%)</th>
<th>ICC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventral striatum</td>
<td>15.8 ± 1.0</td>
<td>16.0 ± 1.1</td>
<td>3.7 ± 0.6</td>
<td>0.98</td>
</tr>
<tr>
<td>Pre-commissural dorsal caudate</td>
<td>18.3 ± 2.1</td>
<td>17.6 ± 1.1</td>
<td>4.4 ± 1.2</td>
<td>0.96</td>
</tr>
<tr>
<td>Pre-commissural dorsal putamen</td>
<td>19.8 ± 1.2</td>
<td>18.8 ± 1.2</td>
<td>5.6 ± 1.0</td>
<td>0.96</td>
</tr>
<tr>
<td>Post-commissural caudate</td>
<td>13.1 ± 0.9</td>
<td>13.1 ± 0.9</td>
<td>3.0 ± 0.9</td>
<td>0.99</td>
</tr>
<tr>
<td>Post-commissural putamen</td>
<td>19.7 ± 1.2</td>
<td>19.2 ± 1.2</td>
<td>3.3 ± 0.7</td>
<td>0.99</td>
</tr>
</tbody>
</table>

ICC, intraclass correlation coefficient. Data are mean ± SEM.
measures ANOVA similarly revealed significant main effects for condition ($F(1,13) = 14.2$, $P = 0.002$), region ($F(4,52) = 76.9$, $P < 0.001$) and a significant condition by subdivision interaction ($F(4,52) = 21.2$, $P < 0.001$). Paired-sample t-tests showed significant amphetamine-induced displacement in all subdivisions (Table V), ranging between 8 and 14%. Partial volume corrected data showed almost identical statistical results. Right–left differences in amphetamine-induced displacement was found in the putamen only ($P = 0.007$) with the right side showing greater displacement (19.5%) compared with the left side (12.1%). Analysis of striatal subdivisions revealed that the left precommissural dorsal putamen showed greater displacement (15.4%) than the right side (11.8%), while the right postcommissural caudate had greater displacement (10.3%) than the left side (5.6%).

Correlations between amphetamine-induced dopamine release and changes in cognition and mood revealed significant negative correlations between changes in the Controlled Oral Word Association Test (CFL) and changes in BPND in the thalamus ($\rho = -0.9$, $P < 0.001$, corrected for multiple comparisons) and the substantia nigra ($\rho = -0.9$, $P < 0.006$, corrected for multiple comparisons), with greater amphetamine-induced dopamine release being associated with better cognitive performance. No significant correlations were found for other cognitive and mood measures and in other regions. Voxel-wise analysis with SPM and SnPM did not detect correlations between amphetamine-induced dopamine release and cognitive changes in subregions of the lateral temporal cortex.

Plasma amphetamine levels were 62 ± 19.2 ng/ml at ~3-h post administration and 71.5 ± 14 ng/ml at ~4.5 h (Table I).

**AMPT-induced changes**

Repeated-measures ANOVAs in striatal subdivisions and other regions revealed no overall effect of condition or interaction of condition by region. Paired-sample t-tests or Wilcoxon signed ranks tests (two-tailed) confirmed that there were no significant changes in BPND following AMPT treatment in striatal and extrastriatal regions. There was large inter-subject variability in AMPT-induced changes in BPND, with some subjects showing large decreases in binding. Percent change of BPND ranged between −3.8 and 1.1% in striatal and −11.7 to 1.2% in extrastriatal regions. No right/left-sided differences in AMPT-induced change in BPND were observed. Plasma levels of AMPT remained stable over the course of the PET scan. The average plasma AMPT concentration was 20 ± 4.3 µg/ml (range 14–27 µg/ml). Plasma levels of AMPT did not correlate with AMPT-induced change in BPND in any region.

**Correlation between amphetamine and AMPT-induced changes**

There were no significant correlations between amphetamine and AMPT-induced changes in BPND in striatal or extrastriatal regions.

**DISCUSSION**

We examined the test–retest variability of [18F]fallypride and the effects of changes in amphetamine-induced and tonic (with AMPT) dopamine and their relationship on [18F]fallypride binding in both striatal and extrastriatal regions. This study showed that the reproducibility of [18F]fallypride measurement in striatal and extrastriatal regions was excellent. With the exception of the anterior cingulate and colliculi, the average absolute difference in...
such displacement was not due to poor \(^{18}\text{F}\)fallypride measurement, indicating that regional percent change exceeds the test–retest variability. Further, our magnitude of binding for the false discovery rate, presumably because placements did not remain significant after controlling for multiple measures and regions with the false discovery rate (Benjamini and Hochberg, 1995), between amphetamine-induced dopamine release in the thalamus and substantia nigra and change in the controlled oral word association test, a test of phonological verbal fluency and executive function. Verbal fluency involves activation of prefrontal, left temporal, anterior cingulate as well as thalamic regions (Frith et al., 1995) and is associated with dopamine function in striatum and frontal cortex (Lawrence et al., 1998; Rinne et al., 2000). As the thalamus forms part of the fronto-striato-thalamic neuronal circuitry (Alexander and Crutcher, 1990), such a relationship with thalamic dopamine release is plausible, whereas in the substantia nigra the relationship may reflect overall dopamine release. Nevertheless, these regional correlations should be interpreted with caution as the influence of practice on cognitive change cannot be determined. The lack of correlations between regional dopamine release and other cognitive measures may be related to insufficient power due to the small sample size and intersubject variability in amphetamine-induced dopamine release and cognitive change.

Although the sample size was small \((n = 6)\), in contrast to Riccardi et al. (2008), we found no effect of AMPT-induced dopamine depletion on \(^{18}\text{F}\)fallypride binding in both striatal and extrastriatal regions in our sample of healthy volunteers. In the Riccardi et al. study, 71.4 mg/kg AMPT over 26 h resulted in significantly increased \(^{18}\text{F}\)fallypride binding (9–13%) in the caudate, putamen, ventral striatum and substantia nigra. In comparison, the effect of a slightly lower dose of AMPT on \(^{18}\text{F}\)fallypride binding in the current study was variable, showing trends of paradoxical decreases in binding or no change, and large

\[^{18}\text{F}\]fallypride \(\text{BP}_{\text{ND}}\) between test and retest was under 10% for all regions.

Consistent with Riccardi et al. (2006), we found that oral administration of amphetamine 3 h before \(^{18}\text{F}\)fallypride injection significantly reduced radioligand binding to D\(_2\) receptors in both the striatum and extrastriatal regions, with the exception of the thalamus and temporal cortex. Amphetamine-induced release ranged between 7 and 14%, with the greatest displacement occurring in the pre- and postcommissional putamen, substantia nigra and medial orbitofrontal cortex, and smaller displacement in the caudate, ventral striatum, temporal cortex, and thalamus. As expected, the magnitude of this displacement was slightly higher than that reported by Riccardi et al. (2006) using \(^{18}\text{F}\)fallypride and a slightly lower dose (0.43 mg/kg) of oral amphetamine. Dopamine release in the thalamus and temporal cortex (both 7%) was reasonably greater than the 3–4% displacement observed by Riccardi et al. Nevertheless, these displacements did not remain significant after controlling for the false discovery rate, presumably because of intersubject variability. Further, our magnitude of regional percent change exceeds the test–retest variability of \(^{18}\text{F}\)fallypride measurement, indicating that such displacement was not due to poor \(^{18}\text{F}\)fallypride reproducibility.

Although the degree of amphetamine-induced dopamine release was slightly higher than previously reported with \(^{18}\text{F}\)fallypride, the rank order of regional displacement was similar. As in the study by Riccardi et al. (2006), the substantia nigra showed a high level of displacement (13%), which was comparable to that seen in the putamen. Although this is speculative, as discussed by Riccardi et al., such unexpectedly high \(^{18}\text{F}\)fallypride displacement in the substantia nigra may be related to the different proportion of D\(_2\) receptors in the substantia nigra and striatum configured in the high- and low-affinity agonist states with a greater proportion of high-affinity state in the former. Another possible explanation is regional differences in the proportion of D\(_2\) and D\(_3\) receptors (Murray et al., 1994) although there has not been a report that fallypride binds preferentially to D\(_2\) receptors, which exists with high density in substantia nigra.

With the exception of the ventral striatum, the magnitude of \(^{18}\text{F}\)fallypride displacement in striatal subdivisions was similar to the 8–16% displacement of \(^{11}\text{C}\)raclopride following an intravenous dose of 0.3 mg/kg amphetamine (Drevets et al., 2001; Martinez et al., 2003). This similarity demonstrates that a relatively high dose of oral amphetamine (0.5 mg/kg) is just as effective as an intravenous dose in displacing radioligand binding, and further, that a high-affinity D\(_2\) radioligand such as \(^{18}\text{F}\)fallypride, has comparable sensitivity to competition from changes in amphetamine-induced dopamine release as does \(^{11}\text{C}\)raclopride. There are several benefits to administering amphetamine orally rather than intravenously. Most importantly, the oral route should result in fewer side effects, which is critical for subject retention and safety. In addition, the duration between oral administration and radioligand injection allows assessment of neuropsychological processes for determination of possible relationships between regional amphetamine-induced dopamine release and changes in cognition and mood.

In this study, we also examined the relationship between changes in dopamine and cognitive function as previous studies have yielded promising findings (for a review see Cropley et al., 2006), including the study by Riccardi et al. (2006) using \(^{18}\text{F}\)fallypride. Despite examining similar cognitive functions as did Riccardi et al. (2006), we did not replicate their finding of significant correlations between amphetamine-induced dopamine release and change in measures of attention and speed of cognitive processing. However, we did observe significant positive correlations (corrected for multiple measures and regions with the false discovery rate) between amphetamine-induced dopamine release in the thalamus and substantia nigra and change in the controlled oral word association test, a test of phonological verbal fluency and executive function. Verbal fluency involves activation of prefrontal, left temporal, anterior cingulate as well as thalamic regions (Frith et al., 1995) and is associated with dopamine function in striatum and frontal cortex (Lawrence et al., 1998; Rinne et al., 2000). As the thalamus forms part of the fronto-striato-thalamic neuronal circuitry (Alexander and Crutcher, 1990), such a relationship with thalamic dopamine release is plausible, whereas in the substantia nigra the relationship may reflect overall dopamine release. Nevertheless, these regional correlations should be interpreted with caution as the influence of practice on cognitive change cannot be determined. The lack of correlations between regional dopamine release and other cognitive measures may be related to insufficient power due to the small sample size and intersubject variability in amphetamine-induced dopamine release and cognitive change.
intersubject variability. Movement of head was not a cause of the paradoxical decrease because no subject showed significant akathisia, and the PET images were corrected for movement. Our findings also differ from those of previous studies reporting AMPT-induced increases of D₂ radioligand binding in the striatum using [¹²³I]IBZM (+28%) (Laruelle et al., 1997) and [¹¹C]raclopride (+13 to 18.5%) (Verhoef et al., 2001, 2002), and in the temporal cortex with [¹²³I]epidepride (+13%) (Fujita et al., 2000).

The reasons for this discrepancy between our results and those from prior studies are unclear but are probably not related to the dose of AMPT. For example, the resulting steady-state levels of AMPT in plasma in our study (20 ± 4.3 μg/ml) were similar to those of Laruelle et al. (1997) and Fujita et al. (2000). On the other hand, although plasma AMPT levels in the current study were comparable with those in previous studies, our subjects showed relatively small subjective and objective AMPT effects, at least in comparison to a previous AMPT study carried out by this same group (Fujita et al., 2000). In that study, subjects experienced strong AMPT effects, indicated by two withdrawals before radioligand infusion due to akathisia and anxiety, and a greater necessity for treatment of side effects. In comparison, none of the eight subjects in the current study withdrew before starting the PET scan. This apparently weaker AMPT effect in our subjects may have been a result of insufficient central dopamine depletion, despite comparable peripheral AMPT plasma levels to other studies.

A main difference between the current study and previous AMPT radioligand binding studies was that we administered a single oral dose of amphetamine before AMPT administration. Whether this had any effect on subsequent D₂ receptor measurement or on the integrity of the dopamine system is unclear. As the elimination half-life of amphetamine in adults is ~13–14 h (Martinsson et al., 2003), there would be no residual amphetamine 1 week after its administration. The shortest interval between amphetamine and AMPT scans was 2 weeks. However, another possibility is that amphetamine exposure altered the sensitivity of the dopamine system, as was recently shown in a study of healthy males (Boileau et al., 2006). Specifically, that study reported increased dopamine release in the striatum 2 weeks and 1 year following three single doses of oral amphetamine, relative to the initial amphetamine dose (i.e., sensitization).

One limitation in the current and previous studies using [¹⁸F]fallypride (Riccardi et al., 2006, 2008) is using cerebellum as the reference region. If specific binding exists in cerebellum that is affected by dopamine levels as shown for [¹¹C]FLB 457 (Asselin et al., 2007; Montgomery et al., 2007), drug-induced changes in [¹⁸F]fallypride binding would have been underestimated. However, changes of [¹⁸F]fallypride binding in cerebellum were not detected in monkey with amphetamine administration (Slifstein et al., 2004b). Therefore, using a reference tissue model in the fallpride studies is unlikely to have caused underestimation in drug-induced changes. Another limitation is that the four PET, test, retest, amphetamine, and AMPT scans were performed in this fixed order. Although it is preferable to apply a randomized design, particularly to study neuropsychological effects, we performed scans in the fixed order because only limited information was available on prolonged effects of amphetamine and AMPT administration and a primary goal of the current study was to examine whether dopamine levels affect [¹⁸F]fallypride binding. The length of residual effects of amphetamine and AMPT must be carefully studied before applying a randomized design.

In summary, the current study demonstrates good reproducibility of [¹⁸F]fallypride measurements, and confirms the feasibility of measuring amphetamine-induced dopamine release in striatal and most extrastriatal regions using oral amphetamine in healthy subjects. However, contrary to recent observations, our results suggest that [¹⁸F]fallypride with AMPT treatment may be unreliable for estimating tonic or baseline dopamine levels in humans.

**ACKNOWLEDGMENTS**

We thank Jeih-San Liow, PhD and Robert Gladding, CNMT for image processing; PMOD Technologies for providing its image analysis and modeling software; Drs. Tom Cooper and Shan Xie, Analytical Psychopharmacology Laboratories, Nathan Kline Research Institute, for conducting plasma assays for amphetamine determination; Dr. George Anderson, Yale University, for determining AMPT plasma levels; and Robert Gladding, CNMT, and the staff of NIH Clinical Center Nursing Unit and NIH PET Department for the successful completion of the study.

**REFERENCES**

Abi-Dargham A, Rodenheimer J, Printz D, Zee-Ponce Y, Gil R, Kegelis LS, Weiss R, Cooper TB, Mann JJ, Van Heertum RL, Gorman JM, Laruelle M. 2000. Increased baseline occupancy of D₂ receptors by dopamine in schizophrenia. Proc Natl Acad Sci USA 97:8104–8109.


Hoekstra PJ, Anderson GM, Troost PW, Kallenberg CGM, Minderaa
Gunn RN, Lammertsma AA, Hume SP, Cunningham VJ. 1997.
Boileau I, Dagher A, Leyton M, Gunn RN, Baker GB, Diksic M, Benkelfat C. 2006. Modeling sensitization to stimul-
Laruelle M, Martinez D, Slifstein M, Brodt A, Mawlawi O, Hwang DR, Huang Y, Coccaro EF, Kegeles LS, Zavaglia AM, Lar-
ropharmacol 26:270–276.
Service.
Mukherjee J, Yang ZY, Das MK, Brown T. 1995. Fluorinated benzam-
de neuroleptics. III. Development of (S)-[(1-allyl-2-pyrrolidinyl)-
Dawant B, Bauernfeind A, Schmidt D, Kessler R. 2006. Amphetamine-induced displacement of [18F] fally-
pride in striatum and extrastriatal regions in humans. Neuro-
pride in striatum and extrastriatal regions in humans. Neuro-
pharmacology 51:274–283.
tion of baseline dopamine D2 receptor occupancy in striatum and extrastriatal regions in humans with positron emission to-
sure baseline occupancy of neostriatal dopamine D2 receptors by dopamine in vivo in healthy subjects. Neuropharmacology 25:213–223.
Verboeff NP, Hussey D, Lee M, Tauscher J, Papatheodorou G, Wil-
son AA, Houle S, Kapur S. 2002. Dopamine depletion results in increased neostriatal D2 receptor binding. J Neuro-

APPENDIX 3: Reprint of Cropley et al. (in press) (uncorrected proof in Psychiatry Research: Neuroimaging)
Pre- and post-synaptic dopamine imaging and its relation with frontostriatal cognitive function in Parkinson disease: PET studies with $^{[11]}$C]NNC 112 and $^{[18]}$F]FDOPA


aMolecular Imaging Branch, National Institute of Mental Health, Bethesda, MD, USA
bHuman Motor Control Section, National Institute of Neurological Disorders and Stroke, Bethesda, MD, USA
cPET Department, Clinical Center, National Institutes of Health, Bethesda, MD, USA
dSchool of Psychology, Psychiatry and Psychological Medicine, Monash University, Clayton, Victoria, Australia
eBrain Sciences Institute, Swinburne University of Technology, Hawthorn, Victoria, Australia

Received 15 August 2007; received in revised form 12 November 2007; accepted 14 November 2007

Abstract

Frontostriatal cognitive dysfunction is common in Parkinson disease (PD), but the explanation for its heterogeneous expressions remains unclear. This study examined the dopamine system within the frontostriatal circuitry with positron emission tomography (PET) to investigate pre- and post-synaptic dopamine function in relation to the executive processes in PD. Fifteen non-demented PD patients and 14 healthy controls underwent $^{[18]}$F]FDOPA (for dopamine synthesis) and $^{[11]}$C]NNC 112 (for D1 receptors) PET scans and cognitive testing. Parametric images of $^{[18]}$F]FDOPA uptake ($K_i$) and $^{[11]}$C]NNC 112 binding potential (BPND) were calculated using reference tissue models. Group differences in $K_i$ and BPND were assessed with both volume of interest and statistical parametric mapping, and were correlated with cognitive tests. Measurement of $^{[18]}$F]FDOPA uptake in cerebral cortex was questionable because of higher $K_i$ values in white than adjacent gray matter. These paradoxical results were likely to be caused by violations of the reference tissue model assumption rendering interpretation of cortical $^{[18]}$F]FDOPA uptake in PD difficult. We found no regional differences in D1 receptor density between controls and PD, and no overall differences in frontostriatal performance. Although D1 receptor density did not relate to frontostriatal cognition, $K_i$ decreases in the putamen predicted performance on the Wisconsin Card Sorting Test in PD only. These results suggest that striatal dopamine denervation may contribute to some frontostriatal cognitive impairment in moderate stage PD.

© 2007 Elsevier Ireland Ltd. All rights reserved.

Keywords: $^{[18]}$F]FDOPA; $^{[11]}$C]NNC 112; PET; Parkinson disease; Frontostriatal cognition; Dopamine

1. Introduction

Cognitive impairment is frequently observed in patients with Parkinson disease (PD), most commonly...
in tests of executive functioning such as working memory, planning, strategies, attentional set-shifting and concept formation (for review, see Cools, 2006). Alteration of the neuronal loops connecting the frontal cortex, thalamus, and basal ganglia (commonly termed frontostriatal circuitry) are suggested to play a role in the executive dysfunction of PD (Owen, 2004). This notion is largely based on the concept of basal ganglia organization, of which frontostriatal circuits are structurally and functionally segregated into “motor”, “limbic” and “associative” (including prefrontal) domains (Alexander et al., 1986; Alexander et al., 1990).

The neurochemical basis of frontostriatal and cognitive dysfunction in PD (particularly in the early stages) is hypothesized to be linked predominately to dopaminergic dysfunction within neural networks linking dorsal striatum (i.e. dorsolateral putamen and dorsal caudate nucleus) to dorsolateral prefrontal cortex (Owen, 2004; Cools, 2006). In PD, tests sensitive to dorsal frontostriatal dysfunction (so-called executive processes) such as planning and set-shifting were impaired following L-dopa (1-3,4-dihydroxyphenylalanine) withdrawal (Lange et al., 1992; Hayes et al., 1998; Cools et al., 2003) and improved with L-dopa treatment (Bowen et al., 1975; Lange et al., 1993), suggesting a primarily dopaminergic substrate. Further, a cerebral blood flow study in PD patients demonstrated dopaminergic modulation of frontostriatal networks during planning (Cools et al., 2002). While together these studies provide strong evidence linking dopamine with frontostriatal executive processes, the findings do not directly address the locus of the molecular pathology and its relationship to the cognitive dysfunction.

Positron emission tomography (PET) allows direct in vivo assessment of pre- and post-synaptic dopaminergic function in PD. Pre-synaptic markers of dopamine neurons include [18F]FDOPA and dopamine transporter ligands, which are consistently lower in the striatum of PD patients (Heiss and Hilker, 2004). Several PET and SPECT studies have correlated striatal, especially caudate nucleus, dopamine loss with cognitive disturbance in PD (Holthoff-Detto et al., 1997; Marie et al., 1999; Müller et al., 2000; Rinne et al., 2000; Bruck et al., 2001). Recently, [18F]FDOPA has also been assessed in the cortex of PD patients and was reduced in the frontal cortex (Rinne et al., 2000) and in the anterior cingulate (Ito et al., 2002). Reductions of frontal cortical [18F]FDOPA uptake in PD were also associated with impairments in working memory, verbal fluency and suppressed attention (Rinne et al., 2000; Bruck et al., 2005). These findings indicate involvement of both striatal and cortical dopamine depletion in the executive impairment of PD, although the precise relationship with frontostriatal tasks such as planning and set-shifting remains unclear.

Work from experimental animals suggests a critical role for post-synaptic dopamine D1 receptors within the prefrontal cortex in modulating executive processes (Arnsten, 1997, 1998). In humans, examination of D1 receptors in executive processes is limited owing to the lack of selective D1 compounds for human use. Whether D1 receptors are altered in PD is largely unknown. Two PET studies have not shown changes in D1 receptor density in striatum and orbitofrontal cortex of PD patients (Shinoh et al., 1993; Ouchi et al., 1999), but both studies used the D1 ligand [11C]SCH 23390 ($K_D = –0.4$ – 0.14 nM), which has low specific-to-nonspecific ratios (Karlsson et al., 1997). The D1 ligand [11C]NNC 112 also displays high affinity for the D1 receptor ($K_D = 0.18$ nM) but shows greater specific-to-nonspecific binding than [11C]SCH 23390 (Halldin et al., 1998). Increases in [11C]NNC 112 binding in the prefrontal cortex were associated with impairments of working memory in schizophrenia (Abi-Dargham et al., 2002). Whether D1 receptors are associated with frontostriatal cognitive processes in PD is not known.

The purpose of the current study was to investigate the relationship between pre- and post-synaptic dopamine markers within the frontostriatal circuitry and executive function in PD. Specifically, the role of striatal and cortical dopamine function on frontostriatal executive processes in non-demented PD was assessed with [18F]FDOPA (a measure of pre-synaptic dopamine synthesis). [11C]NNC 112 (a marker of post-synaptic D1 receptors), and two frontostriatal cognitive tests (the Stockings of Cambridge planning task and the Wisconsin Card Sorting Test).

2. Methods

2.1. Study population

Fifteen non-demented, moderately impaired patients with idiopathic PD (nine males, six females) and 14 age-matched healthy volunteers (eight males, six females) participated in the study (Table 1). Patients were non-smokers and were free of current medical and neurological disorder not related to PD. No patient met current criteria for major depressive disorder, as assessed with the Structured Clinical Interview for DSM-IV Disorders. Controls were non-smokers, medically and neurologically healthy, and free of current psychiatric illness according to DSM-IV Axis I criteria. All but one patient was being treated for PD with L-dopa.
<table>
<thead>
<tr>
<th>Measure</th>
<th>Parkinson disease</th>
<th>Controls</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>62.1±9.2</td>
<td>61.6±8.0</td>
<td>0.16</td>
<td>0.878</td>
</tr>
<tr>
<td>Education</td>
<td>15.3±2.8</td>
<td>16.8±3.1</td>
<td>1.40</td>
<td>0.174</td>
</tr>
<tr>
<td>Mini-Mental State Exam†</td>
<td>29.1±1.0</td>
<td>29.3±1.0</td>
<td>94.5b</td>
<td>0.646</td>
</tr>
<tr>
<td>Beck Depression Inventory‡</td>
<td>1.8±2.8</td>
<td>0.1±0.3</td>
<td>46.5b</td>
<td>0.003</td>
</tr>
<tr>
<td>Dementia Rating Scale Total</td>
<td>139±5.0</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Motor Unified Parkinson</td>
<td>41.9±10.3</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 1
Participant demographics and clinical measures

Data are mean±standard deviation. Group comparisons performed with an independent samples t-test. *Group comparison performed with Mann–Whitney U non-parametric test. †Mann–Whitney U value. P-values are two-tailed.

2.2. Radiopharmaceutical preparation

The (+)-desmethyl-NNC 112 (1.0 mg per radiolabeling) was obtained from Professor Christer Halldin of the Karolinska Institutet. [11C]NNC 112 was synthesized from [11C]methyl iodide (Halldin et al., 1998) via a captive solvent method using a commercially available radiochemistry ‘loop’ module. The radiochemical purities of all [11C]NNC 112 batches were greater than 99%. [18F]FDOPA was produced using the method of Adam and Jivan (1998) with slight modifications to the purification steps. The radiochemical purities of all [18F]FDOPA batches were greater than 90%.

2.3. Scanning protocol

PET scans were performed on a GE Advance tomograph (GE Medical Systems, WI). Before tracer injection, an 8-min transmission scan for attenuation correction of the brain was performed with a 68Ge rotating pin source. Dynamic emission scans were acquired following an intravenous bolus injection of 379–601 MBq of [18F]FDOPA and 391–766 MBq of [11C]NNC 112 for a total scan time of 90 min (6×30 s, 3×1 min, 2×2 min, 16×5 min). Scans were reconstructed with the filtered-back projection algorithm which resulted in a final image resolution of 7.5 mm full width half maximum. For the [18F]FDOPA scan, all subjects received an oral dose of 200 mg carbidopa, a peripheral inhibitor of aromatic-L-amino-acid decarboxylase (AADC), 1 h before scanning. Administration of L-dopa medication was resumed after termination of the [18F]FDOPA PET scan. One PD patient did not complete the [18F]FDOPA scan due to transient high blood pressure after discontinuing L-dopa medication. For the [11C]NNC 112 scans, PD patients continued their normal medication regime. Approximately half of the subjects in each group underwent the [18F]FDOPA scan first. The average interval between [18F]FDOPA and [11C]NNC 112 scans was 18 days. All subjects received a 1.5 T MRI scan for coregistration and segmentation purposes. Inversion recovery fast gradient recalled-echo (IR-FGRE; TR~12 ms, TE~5 ms, flip angle 20°, voxel size: 0.86×0.86×1.2 mm), fast spin echo (FSE) T2-weighted (TR~3700 ms, TE~101 ms, flip angle 90°, voxel size: 0.43×0.43×5 mm) and fluid attenuated inversion recovery (FLAIR; TR~10,002 ms, TE~140 ms, flip angle 90°, voxel size: 0.86×0.86×5 mm) images were obtained.

2.4. Neuropsychological tests

All subjects were administered the Mini-Mental State Examination (Folstein et al., 1975). We included patients with a score of 24 or greater. The Dementia Rating Scale-2 (Jurica et al., 2001), with a cut-off score of ≥123, was additionally administered to PD patients to evaluate the overall cognitive performance and rule out dementia. Depressive symptoms were evaluated with the Beck Depression Inventory fast screen (Beck
Group comparisons (two-tailed) of demographic, clinical and PET variables were performed using the independent sample t-test for parametric data and the Mann–Whitney U test for non-parametric data. Non-parametric variables were determined by the Shapiro–Wilk normality test. Correlations between neuropsychological measures and frontostriatal regions were performed with Spearman’s rank correlation or Pearson’s correlation coefficient, as appropriate. Multiple comparisons were controlled for with a false discovery rate correction (Benjamini and Hochberg, 1995). This modified Bonferroni procedure involves ordering the $P$ values ($P_{(i)}$) for the number of comparisons made from highest to lowest. Controlling the false discovery rate at 0.05, each $P_{(i)}$ is compared sequentially with 0.05 $i/m$, with $m$ being the number of comparisons made. This step-down procedure is continued until a $P$ value satisfies the constraint, and subsequently all hypotheses below this $P$ value are also rejected. All statistical analyses, except voxel-based comparisons, were performed using SPSS for Windows (SPSS Inc., 1989–2004, Release 13.0).

2.6. Image analysis

2.6.1. Preprocessing and parametric imaging

To correct for head movement during the scan, PET frames of both $[^{11}C]$NNC 112 and $[^{18}F]$FDOPA were realigned to a standard frame using the FLIRT algorithm (Jenkinson and Smith, 2001) and MRI (IR, T2 and FLAIR) was coregistered to an average image of initial frames of each of $[^{11}C]$NNC 112 and $[^{18}F]$FDOPA using Statistical Parametric Mapping (SPM2, The Wellcome Department of Cognitive Neurology, London, UK). Parametric images of PET data were calculated using PMOD 2.65 (pixel-wise modeling computer software; PMOD Technologies Ltd, Adliswil, Switzerland). For $[^{18}F]$FDOPA PET scans, parametric images in which each pixel represents the influx constant $K_i$ (min$^{-1}$) of $[^{18}F]$FDOPA were calculated with the Patlak graphical analysis (Patlak and Blasberg, 1985). For each subject, putamen (target) and occipital cortex (reference) volumes of interest were obtained in the Montreal Neurological Institute stereotaxic space. A Patlak plot of the time–activity curve in putamen was used to determine the start time of the linear segment ($t^*$) of the graph. This same $t^*$ was used for pixel-wise calculations of $K_i$ in all target regions. The slope of the linear segment equals the influx constant $K_i$ and represents the uptake rate constant of $[^{18}F]$FDOPA. For $[^{11}C]$NNC 112 scans, parametric images of binding potential (BP$_{ND}$) and $K_i/K_1$’ relative ligand delivery ($R_1$) were generated using the Multilinear Reference Tissue Model 2 (Ichise et al., 2003). BP$_{ND}$ refers to the ratio at equilibrium of the specifically bound radioligand to that of the nondisplaceable radioligand in tissue (see Innis et al., 2007), while $R_1$ is a measure of radioligand delivery to tissue relative to the reference region. Putamen and cerebellum volumes of interest (obtained in Montreal Neurological Institute space) were used as receptor-rich and reference regions, respectively. All data points were used in the fitting for $[^{11}C]$NNC 112, since Logan plots were fairly linear from early time points in a previous study (Abi-Dargham et al., 2000). Parametric images of $[^{18}F]$FDOPA $K_i$ were coregistered to $[^{11}C]$
NNC 112 space, and subsequently both $[^{18}F]$FDOPA and $[^{11}C]$NNC 112 parametric images were spatially normalized to a custom template of the study sample created from the $[^{11}C]$NNC 112 $R_t$ parametric images.

2.6.2. Partial volume correction

Because the thickness of cortical gray matter is only a few millimeters, PET data are a mixture of gray and white matter. We applied partial volume correction to $[^{18}F]$FDOPA PET to minimize the white matter influence on the gray matter signal. $[^{11}C]$NNC 112 data also underwent partial volume correction for purposes of comparison. Partial volume correction was performed using three segments (gray matter, white matter, and cerebrospinal fluid) of MRIs (Müller-Gartner et al., 1992) created from IR, T2 and FLAIR images using SPM2 and coregistered to PET using the previously determined PET/MRI transformation parameters. Binary mask images for gray and white matter were smoothed with a 10-mm Gaussian filter. Gray matter pixels were corrected for spill-out of activity and for spill-in of activity from white matter. To do this, white matter activity was subtracted from the uncorrected image and divided by the smoothed gray matter image. Pure white matter activity was estimated by extrapolating with linear regression the activity values of pixels with a white matter probability greater than 99% (Giovacchini et al., 2004).

The activity that was left was thresholded so there was at least a 20% probability for the pixel to belong to gray matter. PMOD was used to perform partial volume correction. Parametric images of corrected $[^{18}F]$FDOPA $K_t$ and $[^{11}C]$NNC 112 $BP_{ND}$ were created as described above.

2.6.3. Volume of interest analysis

Volumes of interest within the frontostriatal circuitry were applied to $K_t$ and $BP_{ND}$ parametric images normalized to the study sample template. Striatal volumes were defined bilaterally on the caudate nucleus and putamen of a mean image of the study sample’s spatially normalized MRI. Extrastriatal volumes were taken from the anatomical labeling template (Tzourio-Mazoyer et al., 2002) and were defined on the superior, middle and inferior (triangular) lateral frontal gyri and thalamus. Independent sample $t$-tests were performed to compare average $K_t$ and $BP_{ND}$ of patients and controls. Correlations between neuropsychological variables and $K_t$ and $BP_{ND}$ were assessed with Spearman’s or Pearson’s correlation.

2.6.4. SPM analysis

Voxel-based statistical analysis of parametric $K_t$ and $BP_{ND}$ images were performed using SPM2. An isotropic 10-mm Gaussian kernel was used to smooth normalized parametric images. As $K_t$ and $BP_{ND}$ values are quantitative, all SPM analyses were performed without global normalization. Between-group comparisons of $K_t$ and $BP_{ND}$ at the voxel level were performed using a two-sample $t$-test. Analyses testing the correlation between neuropsychological score and $K_t$ or $BP_{ND}$ values were performed with a regression analysis. A false discovery rate of $P < 0.05$ (voxel-level) was considered significant. Because $[^{18}F]$FDOPA data in extrastriatal areas are contaminated by white matter (see below), $K_t$ analyses were done using small-volume correction, i.e. restricted to the striatum. The striatal mask was created from the average $[^{18}F]$FDOPA parametric image of healthy subjects. Covariate analyses of $BP_{ND}$ were made on the whole brain parametric images to explore possible correlations outside the frontostriatal network. Voxel-wise analysis was not performed on partial volume corrected data.

3. Results

3.1. Demographic and neuropsychological data

PD patients did not significantly differ from controls in age, education or Mini-Mental State Examination score (Table 1). Although PD patients reported significantly more symptoms of depression on the Beck Depression Inventory fast screen, the mean score indicated mild depressive symptoms, and no patients were clinically depressed. Furthermore, there were no significant correlations (Spearman’s, two-tailed) between the Beck Depression Inventory and PET and cognitive measures in PD patients. Patients did not significantly differ in performance from controls on any of the neuropsychological measures, although they did not complete as many Wisconsin categories as controls (Table 1). Cognitive performance of PD patients was variable, consisting of both high- and low-performing individuals. Six PD patients (40%) were identified as being cognitively impaired (defined as falling within the 5th percentile of the cognitive test based on normative data) on at least one neuropsychological measure.

3.2. $[^{18}F]$FDOPA uptake

3.2.1. Striatal $[^{18}F]$FDOPA uptake

The mean $K_t$ in putamen and caudate was significantly decreased in PD patients compared with controls (Table 2) with both SPM and volume of interest analysis. In patients, the putamen showed lower $K_t$ values than the caudate nucleus. The $[^{18}F]$FDOPA influx
constant was reduced in PD patients by 70% in the putamen (S.D. =0.04, range: 64–80%) and by 36% in the caudate nucleus (S.D. =0.11, range: 19–56%). PD patients showed lateralized differences in striatal $K_i$, which was significantly lower (paired $t$-test, $t=3.7$, df=13, $P=0.003$,) in striata (putamen and caudate) contralateral to the side of the body with the initial presentation of symptoms in all but two patients.

3.2.2. $[18F]$FDOPA uptake in extrastriatal regions

$[18F]$FDOPA $K_i$ values in extrastriatal regions were considerably lower than in striatum. $K_i$ values were unexpectedly higher in cerebral white matter than adjacent gray matter regions (Fig. 1). Values were approximately two- to three-fold higher in white matter than in frontal gyrus regions. Unlike the asymmetry in striata, $K_i$ in frontal cortex of patients was not related to the side of the body showing initial symptoms.

3.2.3. Partial volume correction of $[18F]$FDOPA PET

Minimizing the influence of white matter data by partial volume correction decreased $K_i$ and increased inter-subject variability in all frontal cortical regions studied. Partial volume correction decreased $K_i$ by approximately 45% and increased COV (S.D./mean) by 45 to 115% in PD patients (Table 3), and decreased $K_i$ by a similar amount (42%) and increased COV by 12 to 55% in controls. Partial volume correction typically increases gray matter PET values, because the signal or variable of interest is typically higher in gray than in white matter. Partial volume correction decreased gray matter $K_i$ values because of greater $K_i$ in white matter. Because of the unreasonably high $K_i$ values in white matter and small and variable $K_i$ values with partial volume correction, $[18F]$FDOPA analyses were only conducted in the striatum.

3.3. $[11C]$NCC 112 binding

Between-group SPM and volume of interest analysis showed no significant differences or trends in regional

Mean±S.D. $[18F]$FDOPA influx constant ($K_i \times 10^{-3} \text{ min}^{-1}$).

Table 2

<table>
<thead>
<tr>
<th>Region</th>
<th>Parkinson disease</th>
<th>Controls</th>
<th>$t$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caudate</td>
<td>7.1±1.2</td>
<td>11.1±1.1</td>
<td>9.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Putamen</td>
<td>3.4±0.5</td>
<td>11.3±1.3</td>
<td>21.5</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Group comparison performed with an independent sample $t$-test.

![Image](image-url)
[11C]NNC 112 BPND between PD patients and controls (Table 4). Patients showed only a weak trend of lower BPND values with the smallest false discovery rate-corrected P value of 0.16 in the right median cingulate. Striatal BPND was approximately seven-fold higher than that in frontal regions, which is consistent with the known distribution of D1 receptors in brain (De Keyser et al., 1988; Hall et al., 1994). A mean parametric image of [11C]NNC 112 BPND in healthy subjects illustrates markedly higher BPND in striatal than in extrastriatal regions (Fig. 2). Patients did not show marked asymmetry of BPND in striatum. BPND values in striata and in frontal cortex of patients were not related to the side of body showing initial symptoms. Partial volume correction of [11C]NNC 112 increased BPND values 2.5 fold and decreased inter-subject variability (COV) by 50%. [11C]NNC 112 BPND values were markedly lower across brain regions in our subjects than in control subjects of a previous PET study using [11C]NNC 112 (Abi-Dargham et al., 2002).

### 3.4. Correlational analyses

For [11C]NNC 112, there were no significant correlations between neurocognitive scores and BPND in PD and controls with volume of interest and SPM analysis. For D1 receptors, the maximum correlation was observed between BPND in the caudate of healthy controls and Stockings of Cambridge perfect solutions (Pearson’s r=0.67, P=0.013), although this did not survive multiple comparison correction. For [18F] FDOPA, correlations were restricted to only the striatum. With volume of interest analysis, PD patients showed a significant positive correlation between Ki in the putamen and number of categories achieved on the Wisconsin Card Sorting Test (Spearman’s rho=0.69, P=0.006) (Fig. 3). SPM with small-volume correction was applied to detect correlations in striatal subdivisions, which would have been missed in the VOI analysis. The SPM analysis did not show significant correlations. No other correlations were found with other neuropsychological measures or in the caudate. Disease severity indices and age were not related to neuropsychological measures in PD, and partial correlations with age as the

### Table 3
Effect of partial volume correction on frontal [18F]FDOPA influx constants in Parkinson disease patients

<table>
<thead>
<tr>
<th>Region</th>
<th>K_i no PVC</th>
<th>COV</th>
<th>K_i PVC</th>
<th>COV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superior frontal gyrus</td>
<td>1.00±0.27</td>
<td>0.27</td>
<td>0.57±0.33</td>
<td>0.58</td>
</tr>
<tr>
<td>Middle frontal gyrus</td>
<td>0.88±0.23</td>
<td>0.26</td>
<td>0.54±0.27</td>
<td>0.50</td>
</tr>
<tr>
<td>Inferior frontal gyrus</td>
<td>1.30±0.26</td>
<td>0.20</td>
<td>0.70±0.20</td>
<td>0.29</td>
</tr>
</tbody>
</table>

Mean±S.D. [18F]FDOPA influx constant (K_i, 10^−3 min⁻¹) in PD patients before and after partial volume correction (PVC). COV=S.D./mean.

### Table 4
[11C]NNC 112 binding potential from volume of interest analysis

<table>
<thead>
<tr>
<th>Region</th>
<th>Parkinson disease</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caudate</td>
<td>1.98±0.38</td>
<td>2.03±0.36</td>
</tr>
<tr>
<td>Putamen</td>
<td>2.35±0.39</td>
<td>2.18±0.38</td>
</tr>
<tr>
<td>Thalamus</td>
<td>0.36±0.99</td>
<td>0.35±0.11</td>
</tr>
<tr>
<td>Superior frontal gyrus</td>
<td>0.26±0.10</td>
<td>0.29±0.09</td>
</tr>
<tr>
<td>Middle frontal gyrus</td>
<td>0.32±0.11</td>
<td>0.34±0.08</td>
</tr>
<tr>
<td>Inferior frontal gyrus</td>
<td>0.33±0.11</td>
<td>0.37±0.10</td>
</tr>
</tbody>
</table>

No significant differences in any region.
control variable produced almost identical statistical results. Volume of interest and SPM analysis showed no significant correlations between striatal $K_i$ and neurocognitive scores in healthy controls.

4. Discussion

In this sample of non-demented PD patients, we found no differences in dopamine D$_1$ receptor density in fronto–striatal–thalamic regions and no overall difference in frontostrriatal cognitive performance. Variability in performance in PD patients on a task reliant on the integrity of the frontostrriatal circuitry was associated with dopamine loss in the putamen. D$_1$ receptor density did not significantly correlate with cognitive performance on frontostrriatal tests. [$^{18}$F]FDOPA uptake values in white matter were erroneously higher than those in gray matter, which casts significant doubt on the validity of cortical dopamine synthesis measurements with [$^{18}$F]FDOPA.

4.1. Pre-synaptic dopamine synthesis and questionable measurement of cortical [$^{18}$F]FDOPA

Consistent with over two decades of research, our PD patients showed reduced $K_i$ in striatum, with greater loss in the putamen than in the caudate nucleus. Unexpectedly however, we found higher [$^{18}$F]FDOPA $K_i$ in white than in adjacent gray matter, which is unreasonable since aromatic amino-acid decarboxylase (AADC) is minimally present in white matter. Over a decade of published [$^{18}$F]FDOPA studies using Patlak parametric modeling with a reference tissue input have not, to our knowledge, reported this phenomenon. One article has shown an [$^{18}$F]FDOPA parametric image with apparently greater $K_i$ in white matter (Nagano et al., 2000), although this was not mentioned. This error in quantification of the cortical [$^{18}$F]FDOPA signal was possibly caused by slower washout of radioactivity from white than gray matter. The reference region (occipital cortex) contains both gray and white matter, and its kinetics would be different from that of either frontal gray or white matter. Such a discrepancy with the reference region would violate an assumption of the Patlak and Blasberg (1985) model that the ratio of activity in nondisplaceable compartments in target compared with reference regions should be constant after $t^*$. We found that the time-activity curves were markedly different between white matter and occipital cortex, which violated this assumption and caused $K_i$ values to be erroneously greater in white matter. Removal of white matter from PET images with partial volume correction to circumvent this model violation actually reduced gray matter $K_i$ and increased intersubject variability. Instead of increasing the specific signal in gray matter, as occurred with [$^{11}$C]NNC 112, the signal became considerably smaller and noisier after partial volume correction, making it vulnerable to statistical noise. Therefore, [$^{18}$F]FDOPA $K_i$ values in extrastriatal regions are unreliable and did not undergo further between-group and correlational analyses.

Our finding of greater $K_i$ in white than adjacent gray matter places significant doubt on the validity and interpretation of cortical [$^{18}$F]FDOPA with reference input models. Six studies have reportedly measured cortical [$^{18}$F]FDOPA uptake in PD patients (Rakshi et al., 1999; Rinne et al., 2000; Kaasinen et al., 2001; Ito et al., 2002; Bruck et al., 2005) or normal elderly controls (Nagano et al., 2000) using cerebellum or occipital cortex as reference regions. Such studies have reported increases (Rakshi et al., 1999; Kaasinen et al., 2001; Bruck et al., 2005) and decreases (Rinne et al., 2000; Ito et al., 2002) in $K_i$ in cortex, which has been interpreted as reflecting either increased or decreased dopamine synthesis. Although parametric images were analyzed, most of these studies presented [$^{18}$F]FDOPA images by summing up data obtained during the entire scan. Please note that such images do not reflect $K_i$ because images at early time points reflect mainly blood flow. Since early images have greater activity than late images, a large portion of the summed up images reflect merely blood flow but not the metabolism of [$^{18}$F]FDOPA to [$^{18}$F]-dopamine. We question whether [$^{18}$F]FDOPA gives a specific PET signal and is meaningful in cortex for the following reasons: (1) Comparisons of $K_i$ in gray and white matter are unreasonable and may be due to a model violation. (2) DOPA decarboxylase activity is very low in frontal cortex of human brain tissue and activity ratio of cortex/caudate is 1% (Mackay et al., 1978). Our ratio of frontal cortex/caudate $K_i$ after partial volume correction in healthy controls was high, with a value of 4%. Previous studies without partial volume correction have shown unrealistically high cortex/caudate ratios of $K_i$ in controls, ranging between 10 and 30% (Rakshi et al., 1999; Nagano et al., 2000; Rinne et al., 2000; Kaasinen et al., 2001; Ito et al., 2002; Bruck et al., 2005), suggesting that [$^{18}$F]FDOPA measurements are not specific to DOPA decarboxylase activity. By not applying partial volume correction, in other words, leaving greater influence from white matter data, frontal cortex/caudate $K_i$ in the current study became 8%, which is close to the ratios reported in previous studies. (3) Tyrosine hydroxylase, the first step dopamine-synthesizing enzyme, and AADC do not
coexist in neurons in human cingulate cortex (Ikemoto et al., 1999), suggesting that AADC-only neurons in at least the cingulate are not specific to dopamine. As such, the scientific community should be aware that the cortical $[^{18}F]$FDOPA signal has serious deficiencies.

### 4.2 Post-synaptic dopamine $D_1$ receptors in Parkinson disease

Lack of alteration of dopamine $D_1$ receptors in our PD sample is consistent with previous studies showing no regional differences in $[^{11}C]$SCH 23390 binding in PD (Shinotoh et al., 1993; Ouchi et al., 1999). Ouchi et al. (1999) studied only early (Hoehn and Yahr stages 1 and 2) PD patients while Shinotoh et al. (1993) examined a heterogeneous sample consisting of patients in Hoehn and Yahr stages 1–4 and with disease duration of 6 months to 10 years. Our study found no changes in $D_1$ receptors in PD patients with moderate symptom severity using a different radioligand for measuring $D_1$ receptors in low-density cortical regions. Taken together, these studies suggest that post-synaptic dopamine $D_1$ receptors are not altered in PD, at least in early to moderate stage patients. A problem with these ligands, however, is their affinity to cortical $5-HT_{2A}$ receptors. A very recent study has shown that about 20 to 30% of cortical $[^{11}C]$NNC 112 uptake in humans is to $5-HT_{2A}$ receptors (Stifstein et al., 2007), making $[^{11}C]$NNC 112 almost equivalent to $[^{11}C]$SCH 23390 with regard to $D_1$ receptor selectivity (Ekelund et al., 2007). Development of more selective ligands for $D_1$ receptors is therefore needed to adequately assess changes in cortical $D_1$ receptor expression in PD.

Due to feasibility issues, PD patients remained on their normal dopaminergic medications for the $[^{11}C]$NNC 112 PET scan. This included l-dopa and dopamine $D_2/D_3$ agonists such as pramipexole and amantadine. Although these medications are not known to directly interact with $D_1$ receptors, it is possible that the indirect increases in extracellular dopamine generated by l-dopa may lead to changes in $D_1$ receptors. However, evidence to date suggests that such changes are unlikely. For example, no changes in $D_1$ receptors have been found in untreated or drug-naive PD patients (Shinotoh et al., 1993; Ouchi et al., 1999), and treated patients (Shinotoh et al., 1993), suggesting that dopaminergic medication has little effect on $D_1$ receptor PET measurement. Furthermore, $[^{11}C]$NNC 112 binding was unaltered following acute administration of the dopamine agonist, amphetamine, in monkeys (Chou et al., 1999). While long-term l-dopa exposure is likely to downregulate $D_1$ receptors (Turjanski et al., 1997), no evidence of this was noted. Nevertheless, such medication-induced interactions as a potential confound on $[^{11}C]$NNC 112 binding cannot be ruled out and requires clarification by measuring $D_1$ receptor availability on and off l-dopa medication.

Binding potential of $[^{11}C]$NNC 112 was about 65–75% lower in our study compared with values reported by Abi-Dargham et al. (2002) in their healthy cohort. This discrepancy was possibly due to differences in the methods for obtaining volume of interest data and age-related decline of $D_1$ receptors in human brain (Suhara et al., 1991; Wang et al., 1998), as our subjects were approximately 30 years older than those in the Abi-Dargham study.

### 4.3 Frontostriatal cognitive function in Parkinson disease and association with pre- and post-synaptic dopamine markers

Our sample of moderately severe PD patients did not show overall frontostriatal cognitive impairment in comparison with elderly controls. Previous studies have reported impairments on the Wisconsin Card Sorting Test (Bowen et al., 1975; Taylor et al., 1986; Brown and Marsden, 1988; Canavan et al., 1989; Paolo et al., 1995) and Tower of London type planning tasks (Owen et al., 1992; Dubois and Pillon, 1997) in medicated, non-demented PD patients, and in patients with a moderate severity of symptoms (Brown and Marsden, 1988; Owen et al., 1992). Our PD patients were tested in an “on-state” relative to their medication effectiveness because some tests are affected by motor function. Thus, the administration of dopaminergic medication may have had a facilitating or normalizing effect on their executive processes and contributed to the lack of overall cognitive impairment. Testing patients both on and off dopamine medication would help determine the effect of dopamine treatment on cognitive processes.

Although PD patients showed no overall cognitive impairment, they did show large variability in frontostriatal cognitive performance. Such variability was not associated with $D_1$ receptors in any region. This contrasts with several PET studies reporting associations between executive processes and $D_1$ receptor density in prefrontal cortex and striatum in disorders associated with dopamine dysfunction, such as schizophrenia and Huntington’s disease (Okubo et al., 1997; Lawrence et al., 1998; Abi-Dargham et al., 2002). Although $D_1$ receptors are proposed to play a critical role in cognitive stability of prefrontal neural networks (Durstewitz et al., 2000; Bilder et al., 2004), as required in maintenance tasks or sustained attention, they may not be critical for...
‘plasticity’ or cognitive flexibility, processes which were largely required in the current study. Whether D2 receptors, which may be more important for ‘resetting/updating’ or behavioral switching functions (see Bilder et al., 2004; Cools, 2006), are associated with planning and set-shifting performance in PD patients remains to be seen.

In contrast, impairment in a measure of executive function (Wisconsin categories achieved) in PD patients was associated with pre-synaptic dopamine loss in the putamen but not the caudate nucleus, a relationship not due to age or disease severity. This relationship was observed even after correction of the false discovery rate (Benjamini and Hochberg, 1995), which is notable as many previous studies reporting similar relationships have not adequately controlled for multiple comparisons. Such an association between putamen $K_I$ and Wisconsin performance is consistent with several PET and SPECT studies showing associations between striatal (particularly caudate nucleus but also putamen) dopamine loss and memory, attention and executive impairment in PD (Holthoff-Detto et al., 1997; Marie et al., 1999; Müller et al., 2000; Rinne et al., 2000; Bruck et al., 2001; Duchesne et al., 2002), suggesting that striatal dopamine depletion in PD may contribute to frontostriatal cognitive impairment. Given the assertion that a “cognitive” loop connects areas of the dorsal prefrontal cortex to the dorsal striatum (including the dorsal caudate and dorsolateral putamen) (Alexander et al., 1986; Alexander et al., 1990), it is surprising that dopamine synthesis in the caudate nucleus was not associated with performance on the Wisconsin Card Sorting Test. While the observed relationship with the putamen fits with the proposed dorsal cognitive loop, the findings are contrary to several animal and human studies reporting an association between caudate nucleus dopamine function and set-shifting ability (Roberts et al., 1994; Marie et al., 1999). Nevertheless, the Wisconsin test reflects executive processes other than attentional set-shifting. A recent functional imaging study suggests that the putamen may play a critical role in the execution stage of a set-shift (Monchi et al., 2006). While it is possible that dopamine synthesis in the putamen may also be associated specifically with the execution stage of the card sorting task, our study does not allow us to delineate specific components of the Wisconsin test that may be modulated by putaminal dopamine synthesis.

Acknowledgements

We thank Jeih-San Liow, Ph.D., for image processing; Hiroto Kuwabara, M.D., Ph.D., and Karen Berman, M.D., for discussion of [18F]FDOPA measurement; PMOD Technologies for providing its image analysis and modeling software; and Robert Gladding, CNMT, and the staff of the PET Department for the successful completion of the study. This research was supported in part by the Intramural Program of NIMH (project number Z01-MH-002852-01).

References


APPENDIX 4: Reprint of Cropley et al. 2006b (Journal of Nuclear Medicine)
Whole-Body Biodistribution and Estimation of Radiation-Absorbed Doses of the Dopamine D₁ Receptor Radioligand ¹¹C-NNC 112 in Humans

Vanessa L. Cropley, BSc¹; Masahiro Fujita, MD, PhD¹; John L. Musachio, PhD¹; Jinsoo Hong, MS¹; Subroto Ghose, MD, PhD¹; Janet Sangare, C-RNP¹; Pradeep J. Nathan, PhD²; Victor W. Pike, PhD¹; and Robert B. Innis, MD; PhD¹

¹Molecular Imaging Branch, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland; and ²Department of Physiology, Monash Centre for Brain and Behaviour, Monash University, Clayton, Victoria, Australia

The present study estimated radiation-absorbed doses of the dopamine D₁ receptor radioligand [¹¹C](-8-chloro-5-(7-benzofuranyl)-7-hydroxy-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepine) (NNC 112) in humans, based on dynamic whole-body PET in healthy subjects. Methods: Whole-body PET was performed on 7 subjects after injection of 710 ± 85 MBq of [¹¹C]-NNC 112. Fourteen frames were acquired for a total of 120 min in 7 segments of the body. Regions of interest were drawn on compressed planar images of source organs that could be identified. Radiation dose estimates were calculated from organ residence times using the OLINDA 1.0 program. Results: The organs with the highest radiation-absorbed doses were the gallbladder, liver, lungs, kidneys, and urinary bladder wall. Biexponential fitting of mean bladder activity demonstrated that 15% of activity was excreted via the urine. With a 2.4-h voiding interval, the effective dose was 5.7 µSv/MBq (21.1 mrem/mCi). Conclusion: [¹¹C]-NNC 112 displays a favorable radiation dose profile in humans and would allow multiple PET examinations per year to be performed on the same subject.

Key Words: [¹¹C]-NNC 112; PET; D₁ receptor; dosimetry; effective dose

J Nucl Med 2006; 47:100–104

Abnormalities in dopamine have been implicated in several psychiatric and neurodegenerative disorders such as schizophrenia, Parkinson’s disease, attention deficit hyperactivity disorder, and drug dependence. Dopamine D₁ receptors are highly distributed in the brain, with the highest density being found in the striatum and regions of the basal ganglia, followed by such regions of the cerebral cortex as the prefrontal cortex, thalamus, hippocampus, and amygdala (1,2). D₁ receptors in the brain have been implicated in the regulation of motor and cognitive activity such as locomotor behavior, working memory, and executive functions (3–5).

[¹¹C](-8-Chloro-5-(7-benzofuranyl)-7-hydroxy-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepine) (NNC 112) is a high-affinity (dissociation constant, 0.18 nmol/L) dopamine D₁ receptor radioligand that can image low-density D₁ receptors in striatal and extrastriatal regions. [¹¹C]-NNC 112 displays higher specific-to-nonspecific ratios than does an older, commonly used ligand, [¹¹C]-SCH-23390 (6,7), and is highly reliable for PET quantification (7). Radiation-absorbed dose estimates of [¹¹C]-NNC 112 have not been reported; therefore, the present study was performed to obtain human dosimetry estimates of [¹¹C]-NNC 112 based on serial whole-body PET scans of healthy subjects.

MATERIALS AND METHODS
Radiopharmaceutical Preparation
The (+)-desmethyl NNC 112 (6) (1.0 µg per radiolabeling) was obtained from Professor Christer Halldin of the Karolinska Institutet. [¹¹C]-NNC 112, originally reported by Halldin et al. (6), was synthesized in radiolabeled form from [¹¹C]-methyl iodide via a captive solvent method using a commercially available radiochemistry “loop” module. The radiochemical purity of all the [¹¹C]-NNC 112 syntheses was greater than 99%, with an average specific activity of 47.0 ± 12.58 GBq/µmol (1.27 ± 0.34 Ci/µmol).

Subjects
The study was approved by the Radiation Safety Committee of the National Institutes of Health and the Institutional Review Board of the National Institute of Mental Health. Seven healthy volunteers (4 male and 3 female; mean age ± SD, 28 ± 8 y; range, 21–42 y) participated in the study. All subjects were free of current medical and psychiatric illness based on history, a physical examination, routine laboratory tests (including a complete blood count, chemistries, urinalysis, urine drug screening, and HIV and hepatitis B tests), and an electrocardiogram. Furthermore, after completion of the PET scan, standard screening laboratory tests were repeated on every subject.
PET Data Acquisition

Subjects underwent transmission and dynamic emission scans on an Advance tomograph (GE Healthcare). Before tracer injection, transmission scans using 68Ge rods were obtained on 7 segments of 15 cm each (the axial field of view of the Advance scanner) from the head to the upper thigh to permit measured attenuation correction. The duration of each transmission was 3 min. After intravenous bolus administration of 710 ± 85 MBq (19 ± 2 mCi) of 11C-NNC 112, dynamic emission scans of 14 frames were acquired by serial imaging of the body in the 7 contiguous segments. The duration of initial frames was 15 s at each segment, followed by frames of increasing length, with six 3-s intervals to move the bed to the next section and 13 s to move the bed back to the first bed position. The total scanning time approximated 120 min (4 × 0.25, 3 × 0.5, 3 × 1, 3 × 2, and 1 × 4 min for each of 7 body sections from head to mid thigh).

The tomographic PET images were compressed into a single anterior-posterior planar image. Planar images were analyzed instead of tomographic images to facilitate visualization of organs. A previous study in our laboratory (8) demonstrated that analysis of compressed planar images is comparable to that of tomographic images, but with a slight overestimation (i.e., conservative calculation) of organ radiation burden. Images were analyzed using pixcelwise modeling computer software (PMOD 2.55; PMOD Group) on the compressed planar images. Regions of interest were drawn on source organs that could be identified. Large regions of interest were drawn to ensure that all accumulated radioactivity, which consisted of both parent and metabolite radioactivities, in each organ was encompassed. The “remainder of body” was calculated for each time point as the decayed value of all activity in the segment minus activity in the identified source organs.

Residence Time Calculation

At each time point, decayed data of the identified source organs were converted to the fraction of the injected dose by dividing the organ activities by the total injected activity and plotted versus time. The total injected activity was calculated as the entire activity in frame 1 in each of the 7 segments. Time–activity curves (percentage injected dose [%ID] vs. time) for the source organs were created. The area under the time–activity curve for each organ was calculated by the trapezoidal method up to the last data acquisition at 120 min. To be conservative, we calculated the area under the curve from the last data acquisition to infinity by assuming that the decline in radioactivity occurred by only physical decay, without any further biologic clearance. The area under the time–activity curve of source organ from time zero to infinity is equivalent to residence time (h).

Organ Absorbed Dose

The activity overlying the bladder represented the total urinary excretion during the scanning interval. For each subject, decay-corrected cumulative urine activity was fitted with a biexponential curve by limiting the urine activity to no more than 100% of the injected activity, and the total excretion in urine in terms of %ID and biologic half-life was determined. The dynamic bladder model (9) implemented in OLINDA/EXM version 1.0 (10) was used to calculate the residence time of the urinary bladder wall with urine-voiding intervals of 2.4 and 4.8 h. Organ absorbed doses were based on the OLINDA/EXM scheme of a 70-kg man, using the residence time of the source organs calculated above.

RESULTS

The injection of 11C-NNC 112 caused no significant change in heart rate or blood pressure, and no significant effects were observed in blood or urine tests performed approximately 5–30 min after termination of the scan. After injection of 11C-NNC 112, the brain, lungs, liver, heart, spleen, kidneys, small intestine, gallbladder, and urinary bladder were visually identified as organs with moderate to high activity in most subjects (Fig. 1). However, the heart was not visualized in 4 subjects, and the small intestine was not visualized in 2 subjects. For the heart and small intestine, the average time–activity curve and residence time were based on 3 and 5 subjects, respectively. Uptake of 11C-NNC 112 was highest in the lungs, with a peak of 28 %ID occurring at the first frame acquisition. Peak values of the %ID to the liver, brain, heart, kidneys, small intestine, and gallbladder were 22, 7, 5, 4, 4, and 1.4, respectively, and all occurred within 5 min. Figure 2 demonstrates the time–activity curves of the source organs at the average time that they were imaged.

The accumulated activity over the bladder was fitted with a biexponential curve to estimate the fraction of injected activity excreted via this route (Fig. 3). In some cases, the fitting of the biexponential curve did not converge; in these instances, the first 1 or 2 data points were eliminated because there were relatively high activities in surrounding soft tissue, compared with that in the urinary bladder. After this step, the biexponential curve well described the accumulation of radioactivity over the bladder, with a mean $r^2$ value of 0.999. According to this fit, the average fraction excreted via urine was approximately 15%, and biologic half-lives were 0.04 and 1.5 h. In most subjects, at the end of the study, the small intestine showed decay-corrected activity of 9.7 %ID. Because of the short half-life, the rest of the injected activity would have decayed before excretion to the bladder or intestine.

The average residence times for the 7 subjects are shown in Figure 4. From the organ residence times and the percentage of activity excreted via the urine, radiation-absorbed dose was estimated for each subject, with urine-voiding
intervals of 2.4 and 4.8 h. There was only a 2% difference in the dose to the urinary bladder wall at urine-voiding intervals of 2.4 and 4.8 h. The effective doses were 5.71 and 5.77 μSv/MBq, with 2.4- and 4.8-h voiding intervals, respectively. With a 2.4-h voiding interval, the organs with the highest radiation burden (μSv/MBq) were the gallbladder (32.4), followed by the liver (22.2), lungs (16.9), kidneys (16.6), and urinary bladder wall (15.7). Table 1 summarizes organ absorbed dose estimates for humans.

**DISCUSSION**

In the present study, serial whole-body 11C-NNC 112 PET studies were performed on healthy humans to estimate radiation-absorbed doses of 11C-NNC 112. Whole-body imaging of 11C-NNC 112 revealed that this D1 receptor radioligand caused only modest radiation exposure, with an effective dose of 5.7 μSv/MBq. With injected activities of 523–788 MBq (14–22 mCi), which are frequently used in brain-imaging studies, this effective dose of 5.7 μSv/MBq (21.1 mrem/mCi) would yield an effective dose of 2.98–4.49 mSv (0.29–0.46 rem). This would allow multiple PET

**FIGURE 2.** (A) Time–activity curves for 11C-NNC 112 serial PET of lungs and liver. Data are expressed as mean ± SD of 7 subjects and are not corrected for radioactive decay. (B) Time–activity curves for 11C-NNC 112 serial PET of 6 organs. Data are expressed as average of 7 subjects for brain, kidneys, spleen, and gallbladder; average of 5 subjects for small intestine; and average of 3 subjects for heart and are not corrected for radioactive decay. For clarity, SD error bars are not included.

**FIGURE 3.** Decay-corrected cumulative urinary excretion of radioactivity after injection of 11C-NNC 112. Data reflect mean ± SD bladder activity measured by PET in 7 subjects. Solid line reflects biexponential fitting of average bladder activity with PRISM software (version 4.0). Two-phase exponential association was used, defined as $Y = Y_{max1} \times (1 - \exp(-K_1 \times X)) + Y_{max2} \times (1 - \exp(-K_2 \times X))$, where $Y = $ cumulative urinary excretion, $Y_{max1}$ and $Y_{max2} = $ 2 fractions of urinary excretion at the infinite time, $X = $ h, and $K_1$ and $K_2 = $ rate constants. Urine activity was constrained to not exceed 100% of injected activity ($Y_{max1} + Y_{max2} = 1$), and half-times were defined as $\ln(2)/K_1$ and $\ln(2)/K_2$. Asymptote of curve indicates that approximately 15%ID was excreted via urine. Fitting to biexponential curve yielded half-lives of 0.04 and 1.5 h.

**FIGURE 4.** For 7 subjects, average residence times (h) calculated from whole-body planar images of 11C-NNC 112. Values are mean ± SD.
Effective dose equivalent = 9.2 \mu Sv/MBq (33.9 mrem/mCi). Effective dose = 5.7 \mu Sv/MBq (21.1 mrem/mCi).

Dynamic urinary bladder model was used, based on 2.4-h void. Effective dose equivalent = 9.2 \mu Sv/MBq (33.9 mrem/mCi). Effective dose = 5.7 \mu Sv/MBq (21.1 mrem/mCi).

Effective dose equivalent = 9.2 \mu Sv/MBq (33.9 mrem/mCi). Effective dose = 5.7 \mu Sv/MBq (21.1 mrem/mCi).

studies per year to be performed on the same subject. Furthermore, the absence of effects on vital signs (heart rate and blood pressure) or on standard blood and urine tests after 11C-NCC 112 injection indicate that 11C-NCC 112 is also safe from a pharmacologic perspective.

The present study demonstrated that the gallbladder is the organ with the highest radiation burden (32.4 \mu Sv/MBq), followed by the liver, lungs, and kidneys. Because planar images were used instead of tomographic images to improve visualization of organs, the gallbladder activities are likely to have included liver activities, and the dose to the gallbladder may have been overestimated. Because planar images were analyzed and large regions of interest were drawn, the present study may have, in general, slightly overestimated the radiation exposure of source organs, resulting in conservative estimates of organ radiation burden. High uptake of 11C-NCC 112 (peak uptake, 28 %ID) initially occurred in the lungs, contributing to the relatively high radiation burden to this organ. This high activity in the lungs at early times reflects distribution of 11C-NCC 112, although the clearance of 11C-NCC 112 from the lungs was rapid, decreasing to 50% of its peak value within 8 min after injection, based on non–decay-corrected data. Activity from injected 11C-NCC 112 at later times primarily reflects elimination of the tracer via the gastrointestinal and urinary routes. Because the distribution of radioactivity in peripheral organs does not match that of dopamine D1 receptors, and because 11C-NCC 112 was metabolized quickly, most of the activities detected in peripheral organs in this study were likely from radioactive metabolites not binding to the receptor. Biexponential fitting of bladder activity demonstrated that only 15% of total activity was excreted via urine. Although there may be errors in the parameter estimation for the urinary excretion because of the short data acquisition, the impact on dose estimations with a 2.4-h urine interval should be minimal because the data were acquired for 2 h—that is, 83% of the interval. Although uptake of 11C-NCC 112 reached a maximum within 5–10 min for all source organs, clearance of 11C-NCC 112 from the liver, gallbladder, and small intestine was quite slow. In most subjects, at the end of the study, the small intestine showed decayed-corrected activity of 9.7 %ID. Because of the short half-life of 11C (20.4 min), the rest of the injected activity (~75%) would have decayed before excretion to the bladder or intestine.

The short half-life of 11C also raises the question of whether human biodistribution studies of 11C-labeled ligands need to be performed, provided that radiation dose estimates in rodents or nonhuman primates have been completed. Table 2 displays effective dose estimates of 11C-labeled ligands obtained from biodistribution studies on humans. For all the tracers listed in Table 2, the estimated effective dose is quite low, allowing multiple injections of the tracer per year in the same subject. With the exception of 1 study (17), which resulted in a conservative estimation of overall radiation burden from 11C-WAY 100635, the effective dose of the 11C tracers shows modest variation, ranging from 4.3 to 7.0 \mu Sv/MBq. With inclusion of 11C/WAY 100635, the effective dose varies appreciably by a factor of 3.3. This relatively large discrepancy may be due to actual differences in radiation exposure from the different 11C-labeled tracers or, rather, may reflect differences in study design and analysis of data.
CONCLUSION

The dopamine D\textsubscript{1} receptor radioligand 11C-NNC 112 appears to be safe for humans, yielding a relatively modest radiation burden that would permit multiple PET studies per year to be performed on the same subject.

ACKNOWLEDGMENTS

We gratefully acknowledge Peter Herscovitch (chief) and the staff of the PET department for the successful completion of the study. We thank Cyrill Burger, PhD, Piotr Rudnicki, PhD, Krzysztof Mikolajczyk, PhD, and Michal Grodzki, PhD, for providing the PMOD 2.55 software and Jeih-San Liow, PhD, for technical assistance. This research was supported (in part) by the Intramural Research Program of the National Institutes of Health and National Institute of Mental Health.

REFERENCES

APPENDIX 5: Poster presented at “Dopamine 50 Years” symposium in Göteborg, Sweden (May 2007)
INTRODUCTION

Dopamine and cognition

- Dopamine plays a critical role in modulating cognitive processes.
- Studies in monkeys show that dopamine and D2 receptors in the PFC mediate working memory and other cognitive processes subserved by frontostriatal neuronal loops.

Parkinson disease and the dopaminergic system

- Parkinson disease (PD) is a neurodegenerative disorder characterized by alteration of the dopamine system.
- PD patients show impairment in frontostriatal cognitive processes such as planning and attentional set-shifting, which has been linked to the dopamine system.
- It is unclear whether D2 receptors are altered in PD, and to what extent extrastriatal dopaminergic alterations are related to the cognitive deficits of PD.

OBJECTIVES

To examine pre- and post-syntactic dopamine function in striatal and cortical regions of PD and its relation to frontostriatal cognition.

METHODS

- Fifteen non-demented PD patients and 14 healthy controls received [18F]FDOPA and [11C]NNC 112 PET scans. MRI and neuropsychological battery.
- PET scans acquired for 90 min on GE Advance.
- PD patients medication-free for at least 12 h before PET scans acquired for 90 min on GE Advance.
- Partial volume correction of [18F]FDOPA measurement was problematic.
- Assumption violated

RESULTS

Group Differences

- [11C]NNC 112 binding potential in VOI analysis

Table 1. Participant demographics and clinical measures

<table>
<thead>
<tr>
<th>Measure</th>
<th>Parkinson disease</th>
<th>Control</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>63.2±12</td>
<td>65.5±10</td>
<td>0.06</td>
</tr>
<tr>
<td>Education:</td>
<td>15.6±1.3</td>
<td>16.3±1.3</td>
<td>0.07</td>
</tr>
<tr>
<td>MNR:</td>
<td>15.5±1.3</td>
<td>16.3±1.3</td>
<td>0.07</td>
</tr>
<tr>
<td>FDR:</td>
<td>15.5±1.3</td>
<td>16.3±1.3</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Data Analysis

- Generation of parametric images
- [18F]FDOPA: influx constant (Ki) with Patlak® model and cortical as reference region.
- Spatial normalization to AC/PC custom template of study sample.
- Partial volume correction of [18F]FDOPA to minimize white matter issues.
- Group differences in Ki and BP assessed with volume of interest and voxel-wise analysis.
- Correlation between neuropsychological scores and both Ki and BP with SPM and SPSS.

DISCUSSION

In this sample of non-demented PD patients there was:

- No change in striatal and cortical D2 receptor density.
- No change in frontostriatal neuropsychological function.
- Significantly decreased dopamine metabolism in striatum.
- Correlation between WSCST, a measure of executive function, and Ki in the putamen.

Although D2 receptors have been implicated in executive function, our findings suggest that moderate stage PD patients do not have alterations in cortical and striatal D2 receptors to an extent to cause frontostriatal cognitive impairment.

Our finding of an association between putamen Ki and executive function is consistent with several previous PET studies in PD.

Cortical [18F]FDOPA measurement was problematic. Higher K values were found in cortical white than gray matter, which is paradoxical since there are insignificant amounts of AADC in white matter.

This was likely caused by violation of the Patlak reference tissue model. PVC decreased [18F]FDOPA signal and increased inter-subject variability.

In summary, striatal dopamine denervation may contribute to some frontostriatal cognitive impairment in moderate stage PD.