THE EFFECTS OF ELECTROCONVULSIVE THERAPY IN AN ANIMAL MODEL OF PARKINSON’S DISEASE: MECHANISMS OF A POTENTIAL ADJUNCT TREATMENT

by

ELISSA MARIE STROME

B.Sc., Trent University, 1999
M.Sc. The University of British Columbia, 2001

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE STUDIES

(Neuroscience)

THE UNIVERSITY OF BRITISH COLUMBIA

June 2006

© Elissa Marie Strome, 2006
Abstract:

Electroconvulsive therapy (ECT) is a widely used and effective treatment for mood disorders. ECT also appears to have positive effects in Parkinson's disease (PD), improving motor symptoms for several weeks. Some of the most consistent effects of electroconvulsive shock (ECS) in animals are enhancement of both monoamine neurotransmission and neurotrophic factor concentrations in limbic brain regions. We hypothesized that the mechanism of action of ECT in PD is similar to its proposed mechanism of action in depression, more specifically, that ECS in 6-hydroxydopamine (6-OHDA)-lesioned rats will: 1) improve motor behaviour; 2) enhance D₁ and D₃ (without changing D₂) receptor binding; and 3) enhance striatal neurotrophic factor concentrations. We performed several pilot and validation studies to determine the appropriate animal models (non-human primate vs. rat model of PD) and to develop tools to investigate our hypotheses with (autoradiography with positron-emitting tracers and non-pharmacological motor evaluation). To examine our three specific hypotheses, we treated 6-OHDA-lesioned rats with ECS or sham treatment and examined their motor behaviour using two non-pharmacological behavioural tests; the Cylinder Test, which evaluates forelimb function, and the Tapered/Ledged Beam-Walking Test, which examines hindlimb function. After the course of ECS or sham treatment, the animals were sacrificed, and their brains were removed and processed for either dopamine receptor binding or neurotrophic factor concentration. ECS treatment significantly improved hindlimb function, but had no apparent impact on forelimb function. ECS treatment also enhanced striatal D₁ and D₃ receptor binding, without affecting D₂ binding. Finally, repeated ECS treatment decreased brain-derived neurotrophic factor (BDNF) concentrations in the prefrontal cortex (PFC), but increased BDNF in the hippocampus and striatum. Basic fibroblast growth factor (FGF-2) concentrations were increased in the striatum, whereas glial cell line-derived neurotrophic factor protein was significantly decreased in the PFC. This body of work provides the first thorough investigation of the effects of repeated ECS treatment in the 6-OHDA-lesioned rat, showing that it improves motor function, enhances DA neurotransmission via upregulation of the D₁ and D₃ receptors, and increases striatal BDNF and FGF-2, and the results support the continued use and study of ECT as an adjunctive treatment for PD.
Table of Contents:

Abstract .................................................................................................................................................. ii
Table of Contents .................................................................................................................................. iii
List of Tables ......................................................................................................................................... vii
List of Figures ....................................................................................................................................... viii
List of Symbols and Abbreviations ...................................................................................................... x
Acknowledgments ............................................................................................................................... xi
Dedication ............................................................................................................................................... xii
Co-Authorship Statement ...................................................................................................................... xiii

Chapter 1: Introduction
Literature Review ................................................................................................................................. 1
  Parkinson’s Disease .......................................................................................................................... 1
  Electroconvulsive Therapy .............................................................................................................. 1
  Electroconvulsive Shock as an Animal Model of ECT ................................................................. 2
Overview and Objectives .................................................................................................................... 7
Model and Tool Development ............................................................................................................ 8
Hypotheses .......................................................................................................................................... 9
  Working Hypothesis ....................................................................................................................... 9
  Hypothesis 1 .................................................................................................................................. 12
  Hypothesis 2 .................................................................................................................................. 12
  Hypothesis 3 .................................................................................................................................. 13
References ............................................................................................................................................ 15

Chapter 2: Electroconvulsive shock decreases binding to 5-HT\textsubscript{2} receptors in nonhuman primates: an in vivo positron emission tomography study with [\textsuperscript{18}F]setoperone
Preamble ............................................................................................................................................... 25
Introduction ......................................................................................................................................... 25
Materials and Methods ....................................................................................................................... 27
  Subjects .......................................................................................................................................... 27
  ECS Procedure ............................................................................................................................... 27
  PET Procedure ............................................................................................................................... 27
  Data Analysis ................................................................................................................................. 28
  Statistical Analysis ......................................................................................................................... 28
Results ............................................................................................................................................... 29
Discussion .......................................................................................................................................... 34
References ........................................................................................................................................... 38

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preamble</td>
<td>43</td>
</tr>
<tr>
<td>Introduction</td>
<td>43</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>44</td>
</tr>
<tr>
<td>Subjects</td>
<td>44</td>
</tr>
<tr>
<td>5-HT$_2$ Binding: $[^18]F$Setoperone</td>
<td>44</td>
</tr>
<tr>
<td>$[^18]F$Setoperone Standard Curves</td>
<td>45</td>
</tr>
<tr>
<td>$[^18]F$Setoperone Binding Analysis</td>
<td>46</td>
</tr>
<tr>
<td>Resolution of $[^18]F$ Phosphor Imaging</td>
<td>46</td>
</tr>
<tr>
<td>Statistical Analysis</td>
<td>48</td>
</tr>
<tr>
<td>Results</td>
<td>50</td>
</tr>
<tr>
<td>Technical Issues of $[^18]F$ Phosphor Imaging</td>
<td>50</td>
</tr>
<tr>
<td>Fit and Reproducibility of $[^18]F$ Standard Curves</td>
<td>50</td>
</tr>
<tr>
<td>Resolution of $[^18]F$ Phosphor Imaging</td>
<td>50</td>
</tr>
<tr>
<td>Reusability of Tritium-Sensitive Phosphor Screens</td>
<td>53</td>
</tr>
<tr>
<td>Effects of DMI Treatment on 5-HT$_2$ Binding</td>
<td>54</td>
</tr>
<tr>
<td>Treatment and Regional Effects</td>
<td>54</td>
</tr>
<tr>
<td>Comparison of PET and Tritiated Ligands</td>
<td>56</td>
</tr>
<tr>
<td>Discussion</td>
<td>56</td>
</tr>
<tr>
<td>Phosphor Imaging with PET Ligands</td>
<td>60</td>
</tr>
<tr>
<td>References</td>
<td>62</td>
</tr>
</tbody>
</table>

Chapter 4: Evaluation of the integrity of the dopamine system in a rodent model of Parkinson’s disease: small animal PET compared to behavioral assessment and autoradiography

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preamble</td>
<td>65</td>
</tr>
<tr>
<td>Introduction</td>
<td>65</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>67</td>
</tr>
<tr>
<td>Subjects</td>
<td>67</td>
</tr>
<tr>
<td>6-OHDA Lesioning</td>
<td>67</td>
</tr>
<tr>
<td>Tapered/Ledged Beam-Walking Test</td>
<td>67</td>
</tr>
<tr>
<td>MicroPET Imaging</td>
<td>68</td>
</tr>
<tr>
<td>Autoradiography</td>
<td>69</td>
</tr>
<tr>
<td>Statistical Analysis</td>
<td>69</td>
</tr>
<tr>
<td>Results</td>
<td>70</td>
</tr>
<tr>
<td>Discussion</td>
<td>75</td>
</tr>
<tr>
<td>References</td>
<td>78</td>
</tr>
</tbody>
</table>

Chapter 5: Electroconvulsive shock enhances D$_1$ and D$_3$ receptor binding and improves motor behaviour in 6-OHDA-lesioned rats

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preamble</td>
<td>82</td>
</tr>
<tr>
<td>Introduction</td>
<td>83</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>84</td>
</tr>
<tr>
<td>Subjects</td>
<td>84</td>
</tr>
<tr>
<td>6-OHDA Lesioning</td>
<td>84</td>
</tr>
</tbody>
</table>
Chapter 6: The effects of electroconvulsive shock on GDNF, BDNF, and FGF-2 concentrations in the 6-OHDA-lesioned rat brain

Preamble.................................................................................... 101
Introduction............................................................................. 101
Materials and Methods.......................................................... 102
  Subjects.............................................................................. 102
  6-OHDA Lesioning............................................................... 102
  Cylinder Use Asymmetry Test............................................ 103
  Electroconvulsive Shock Treatment................................... 103
  Tissue Processing............................................................... 103
  GDNF and BDNF ELISA.................................................... 104
  FGF-2 ELISA..................................................................... 104
  Data Analysis.................................................................... 104
Results.................................................................................. 105
  Cylinder Test.................................................................... 105
  GDNF ELISA..................................................................... 105
  BDNF ELISA..................................................................... 105
  FGF-2 ELISA..................................................................... 105
Discussion............................................................................. 110
References............................................................................ 114

Chapter 7: General Discussion

Preamble.................................................................................... 120
Model and Tool Development.............................................. 120
  Model Development.......................................................... 120
  Tool Development............................................................. 122
The Effects of Repeated ECS on the DA System in Parkinsonian Rats............................................. 123
  Status of Working Hypothesis.......................................... 123
  Hypothesis 1..................................................................... 123
  Hypothesis 2..................................................................... 124
Hypothesis 3........................................................................125
Synthesis of the Findings.....................................................126
Limitations of the Studies....................................................126
Directions for Future Study................................................130
Significance of the Findings to PD.................................132
Conclusions......................................................................133
References.......................................................................134

Appendix A: The use of anaesthesia for repeated ECS treatment in the rat
Introduction........................................................................142
Materials and Methods....................................................142
Results............................................................................143
Discussion.......................................................................147
References.....................................................................148

Appendix B: The effects of repeated ECS under ketamine anesthesia on BDNF mRNA expression
Introduction........................................................................150
Materials and Methods....................................................150
Results............................................................................150
Discussion.......................................................................150
References.....................................................................152
List of Tables:

Table 1.1: Effects of ECT in Parkinson’s disease or parkinsonism ........................................ 3

Table 1.2: Changes in DA receptors following repeated ECS .................................................. 4

Table 1.3: Brief review of the literature on the effects of repeated ECS on the neurotrophic factors BDNF, FGF-2, and GDNF .............................................................. 6

Table 2.1: Polynomial contrasts to determine the pattern of the changes in 5-HT$_2$ binding after a course of ECS ........................................................................................................... 32

Table 3.1: Characteristics of the point source series used to create Figure 3.2 ......................... 48

Table 3.2: Determination of the spatial resolution of the Multisensitive phosphor screens .... 52

Table 3.3: Decreased 5-HT$_2$ binding after chronic treatment with DMI detected using [$^{18}$F]setoperone ...................................................................................................................... 55

Table 3.4: Correlation between [$^{18}$F]setoperone and [$^3$H]ketanserin binding ....................... 55

Table 4.1: Comparison of percent lesion after unilateral infusion of 20 μg (High Dose) or 2 μg (Low Dose) 6-OHDA, as measured by striatal [$^{11}$C]DTBZ binding in vivo by microPET and in vitro with autoradiography ................................................................. 71
List of Figures:

Figure 1.1: Working hypothesis to explain the mechanism of action of ECT in PD .................10

Figure 2.1: Relationship between seizure threshold and seizure length across the course of ECS ..........................................................29

Figure 2.2: [18F]Setoperone binding to 5-HT2 receptors throughout the cortex in seven adult male rhesus monkey at baseline (pre-ECS), 24 hours, 1 week and 4-6 weeks after the last ECS session ..................................................31

Figure 2.3: Percent change in [18F]setoperone binding to 5-HT2 receptors ........................................33

Figure 3.1: The same [18F]standard displayed three different ways, illustrating the spillover effect ..................................................................................................................................................47

Figure 3.2: Point sources printed on paper with [18F] ink ........................................................................49

Figure 3.3: Fit of the curves derived from the [18F] standards .................................................................51

Figure 3.4: Spillover correction decreases the variability of the slopes of standard curves from different days ........................................................................................................................................52

Figure 3.5: Background subtraction corrects for repeated use of tritium sensitive phosphor screens ...............................................................................................................................................53

Figure 3.6: Background subtraction compensates for accumulation of moisture by tritium-sensitive phosphor screens for up to three months after first opening ......................................................................................54

Figure 3.7: Decreased 5-HT2 binding after chronic DMI treatment .........................................................57

Figure 3.8: Autoradiographic detection of decreased 5-HT2 binding using phosphor imaging and two different radioligands ........................................................................................................................................58

Figure 4.1: The tapered/ledged beam-walking test apparatus .........................................................................68

Figure 4.2: Relationships between the three measures of striatal DA integrity ........................................72

Figure 4.3: TB test scores for each hindlimb in individual animals after severe (A-G) or mild (H-M) unilateral 6-OHDA lesioning ..................................................................................................................................73

Figure 4.4: Coronal images of striatal [11C]DTBZ binding through the head and brain with microPET (left) and in brain only with autoradiography (right) ................................................................................74

Figure 5.1: Forelimb use asymmetry scores before lesioning, and before and 48 h after repeated ECS or sham treatment ...............................................................................................................................................88

Figure 5.2: TB test data for the hindlimb contralateral to the lesion on the narrow section of the beam in ECS- and sham-treated rats before and 48 h after treatment ........................................................................88
Figure 5.3: DA receptor binding values in the dorsal and ventral striatum in ECS- and sham-treated rats ........................................................................................................90-91

Figure 6.1: Forelimb use asymmetry scores before lesioning, and before and 48 h after repeated ECS or sham treatment .........................................................................................106

Figure 6.2: The effects of repeated ECS treatment on GDNF concentrations in the brain ......107

Figure 6.3: The effects of repeated ECS treatment on BDNF concentrations in the brain ......108

Figure 6.4: The effects of repeated ECS treatment on FGF-2 concentrations in the brain ......109

Figure 7.1: Summary of the results of this body of work in the context of the working hypothesis ........................................................................................................................127

Figure 7.2: DA and BDNF synergism within the striatum after ECS treatment ..................129

Figure A.1: Mean charge dose (± SEM) required to elicit a seizure during the course of ECS under one of four conditions ..............................................................................................144

Figure A.2: Mean seizure length (± SEM) during the course of ECS under one of four conditions ..........................................................................................................................144

Figure A.3: Probability of eliciting a seizure with tonic hindlimb extension (THLE) under four different conditions as the course of ECS progressed ................................................145

Figure A.4: Repeated ECS treatment under ketamine increases frontal cortex [18F]setoperone binding ...........................................................................................................................145

Figure A.5: Frontal cortex 5-HT2 receptor binding in rats treated with ECS or sham under ketamine anaesthesia as measured by [18F]setoperone .......................................................................146

Figure B.1: The effects of repeated ECS treatment under ketamine anaesthesia on BDNF mRNA expression in the piriform cortex ..................................................................................151
**List of Symbols and Abbreviations:**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-MHPG</td>
<td>3-methoxy-4-hydroxyphenyl-glycol</td>
</tr>
<tr>
<td>5-HT</td>
<td>5-hydroxytryptamine (serotonin)</td>
</tr>
<tr>
<td>6-OHDA</td>
<td>6-hydroxydopamine</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>ANCOVA</td>
<td>analysis of covariance</td>
</tr>
<tr>
<td>BDI</td>
<td>Beck Depression Inventory</td>
</tr>
<tr>
<td>BDNF</td>
<td>brain derived neurotrophic factor</td>
</tr>
<tr>
<td>BP</td>
<td>binding potential</td>
</tr>
<tr>
<td>cAMP</td>
<td>cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>CREB</td>
<td>cAMP response element-binding protein</td>
</tr>
<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
</tr>
<tr>
<td>DA</td>
<td>dopamine</td>
</tr>
<tr>
<td>DAT</td>
<td>dopamine transporter</td>
</tr>
<tr>
<td>DBS</td>
<td>deep brain stimulation</td>
</tr>
<tr>
<td>DLU</td>
<td>digital light units</td>
</tr>
<tr>
<td>DMI</td>
<td>desipramine</td>
</tr>
<tr>
<td>DTBZ</td>
<td>dihydrotetrabenazine</td>
</tr>
<tr>
<td>ECS</td>
<td>electroconvulsive shock</td>
</tr>
<tr>
<td>ECT</td>
<td>electroconvulsive therapy</td>
</tr>
<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>FGF-2</td>
<td>basic fibroblast growth factor</td>
</tr>
<tr>
<td>FWHM</td>
<td>full-width at half-maximum</td>
</tr>
<tr>
<td>GABA</td>
<td>γ amino butyric acid</td>
</tr>
<tr>
<td>GDNF</td>
<td>glial cell line-derived neurotrophic factor</td>
</tr>
<tr>
<td>HAM-D</td>
<td>Hamilton Depression Rating Scale</td>
</tr>
<tr>
<td>HVA</td>
<td>homovanillic acid</td>
</tr>
<tr>
<td>ICV</td>
<td>intracerebroventricular</td>
</tr>
<tr>
<td>Kd</td>
<td>receptor affinity</td>
</tr>
<tr>
<td>LID</td>
<td>L-DOPA-induced dyskinesia</td>
</tr>
<tr>
<td>MAP</td>
<td>maximum a posteriori</td>
</tr>
<tr>
<td>MAP kinase</td>
<td>mitogen-activated protein kinase</td>
</tr>
<tr>
<td>MPTP</td>
<td>1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine</td>
</tr>
<tr>
<td>NAcc</td>
<td>nucleus accumbens</td>
</tr>
<tr>
<td>NPY</td>
<td>neuropeptide Y</td>
</tr>
<tr>
<td>PD</td>
<td>Parkinson’s disease</td>
</tr>
<tr>
<td>PET</td>
<td>positron emission tomography</td>
</tr>
<tr>
<td>PFC</td>
<td>prefrontal cortex</td>
</tr>
<tr>
<td>PKA</td>
<td>protein kinase A</td>
</tr>
<tr>
<td>PSF</td>
<td>point-spread function</td>
</tr>
<tr>
<td>ROD</td>
<td>relative optical density</td>
</tr>
<tr>
<td>ROI</td>
<td>region of interest</td>
</tr>
<tr>
<td>SN</td>
<td>substantia nigra</td>
</tr>
<tr>
<td>SNpc</td>
<td>substantia nigra pars compacta</td>
</tr>
<tr>
<td>SPECT</td>
<td>single photon emission computed tomography</td>
</tr>
<tr>
<td>SSRI</td>
<td>selective serotonin reuptake inhibitor</td>
</tr>
<tr>
<td>TB test</td>
<td>tapered/ledged beam-walking test</td>
</tr>
<tr>
<td>TCA</td>
<td>tricyclic antidepressant</td>
</tr>
<tr>
<td>TH</td>
<td>tyrosine hydroxylase</td>
</tr>
<tr>
<td>THLE</td>
<td>tonic hindlimb extension</td>
</tr>
<tr>
<td>UPDRS</td>
<td>Unified Parkinson Disability Rating Scale</td>
</tr>
<tr>
<td>VMAT2</td>
<td>vesicular monoamine transporter</td>
</tr>
</tbody>
</table>

2
Acknowledgements:

I'd like to thank my supervisor, Dr. Doris Doudet, for her support during the course of my graduate studies, as a mentor, friend, and finally colleague. I am grateful for all of the many opportunities that Doris gave me to do good research, and know that she was the key to my success.

Thanks to my mom and dad, for encouraging my independence from a very young age, for teaching me about the importance of family, and for supporting me in everything that I have ever done.

I owe a great debt of gratitude to all of my colleagues at the Pacific Parkinson’s Research Centre and the UBC/TRIUMF PET program. Over the years, I have benefited from the expertise and hard work of so many people; the principal investigators, Tom Ruth, Jon Stoessl, and Vesna Sossi; technicians and technologists, Jessica Grant, Rick Kornelsen, Salma Jivan, Carolyn English, Caroline Williams, Ken Buckley, Siobhan McCormick, and all the staff at the Animal Resource Unit; and my fellow graduate students, Joe Flores and Ivan Cepeda.

Thanks also to the members of my supervisory committee, Drs. Tony Phillips, Cathy Rankin, and Wolf Tetzlaff, and ex officio member Dr. Athanasios Zis, for sharing their expertise, and for their guidance, support, and constructive critique of my research project.

During the course of this body of research, I personally received funding from the Natural Sciences and Engineering Research Council of Canada (NSERC), and from the Michael Smith Foundation for Health Research (MSFHR), and we received funding for the project from the Canadian Institutes of Health Research (CIHR). Those scholarship and research dollars were greatly appreciated, and, I think, well spent.

I'd also like to recognize my research subjects, without whom none of the questions within these pages could have been addressed.

Thanks to Erin, for many years of friendship and stimulating scientific discussions.

Thanks to Carey, for being my kindred spirit.

And of course, I have to thank Dave, for being there.
Dedication:

To my grandfather, whose behaviour inspired my childhood curiosity, and motivated my career choice as an adult.
Co-Authorship Statement:

Much of the work included in Chapters 2-6 of this thesis was performed in collaboration with my research supervisor, mentors, fellow graduate students, and colleagues at the University of British Columbia. Every attempt has been made to give credit where it is due, either by sharing co-authorship in the published manuscripts, or by acknowledging assistance at the end of the manuscripts or in the Acknowledgments section of this thesis. In general, I performed 70-90% of the work required to produce a publishable manuscript independently, including most of the data collection, analysis, and interpretation, and all of the writing, save for comments or suggestions from my collaborators on the draft manuscripts.
Chapter 1

Introduction

Literature Review:

Parkinson's Disease

Parkinson's disease (PD) is a progressive and debilitating neurodegenerative disorder affecting over 100,000 Canadians. The hallmark of the disease is the degeneration of the dopamine (DA)-containing neurons of the substantia nigra pars compacta (SNpc), but other neuropathological symptoms can also be detected at post-mortem, including the formation of Lewy bodies, and the depletion of the other monoamine neurotransmitters serotonin (5-HT) and norepinephrine (reviewed in Lang and Lozano, 1998a). The primary symptoms of PD include tremor, rigidity, bradykinesia (slow movement), hypokinesia (paucity of movement) and postural instability with gait disturbance. The early motor symptoms of PD are treated effectively with the DA precursor L-DOPA in combination with a peripheral metabolism inhibitor (carbidopa), and/or other adjunctive medications such as DA agonists. Within 5-7 years, however, the response to L-DOPA is diminished, and patients experience “wearing-off” or “on-off” phenomena, and/or dyskinesia (abnormal involuntary movements; see Lang and Lozano, 1998b for review). Surgical interventions, either lesioning (e.g. in pallidotomy) or implanting a high frequency stimulator (known as deep brain stimulation, or DBS) in the major basal ganglia output structures are effective as subsequent steps in the treatment course (Walter and Vitek, 2004), but these treatments are not without risk, do not provide indefinite relief, and may introduce further side effects.

With increasing disease severity also come symptoms outside the realm of movement including sleep and cognitive disturbances, as well as affective disorders, predominantly in the form of major depression. Prevalence studies suggest that 30-40% of PD patients suffer from comorbid major depression (Tandberg et al., 1996; Slaughter et al., 2001). Although traditional antidepressants such as tricyclic antidepressants or selective serotonin reuptake inhibitors (SSRIs) are often prescribed to treat depressive symptoms in PD, their use, safety, and efficacy have not been thoroughly investigated. In fact, a recent meta-analysis examining eleven studies concludes that the placebo effect is as strong as the true drug effect after antidepressant treatment for depression in PD (Weintraub et al., 2005). In addition, as in the normal depressed population, some patients may not respond well to antidepressant drugs, and there are concerns about the development of side effects after the use of SSRIs in PD because they can cause movement disorders in and of themselves (Caley, 1997; Gerber and Lynd, 1998). Electroconvulsive therapy (ECT) is a safe, effective, and widely used alternative for all patients who find no relief from their depression after trying several antidepressant drugs, or for those who develop negative side effects.

Electroconvulsive Therapy

ECT has a long and controversial history in psychiatry, and as such, it is often used only as a last resort to treat many psychiatric disorders. Several recent meta-analyses have shown, however, that ECT is more effective at treating depression than antidepressant drugs (UK ECT Review Group, 2003; Kho et al., 2003; Pagnin et al., 2004). It is particularly effective in depression, certain types of schizophrenia, mania, and catatonia (Fink, 2001). ECT is the treatment of choice for depression in pregnant women, and in psychiatric emergencies. There are few contraindications (intracerebral mass or recent myocardial infarction), and it is associated with very low morbidity and mortality. Although the risks associated with ECT are
minimal, the treatment is associated with one consistent side effect, in the form of memory disturbance. While patients do typically experience both retrograde and/or anterograde memory disturbance, the loss is transient and involves only the weeks surrounding the treatment (reviewed in Rami-Gonzalez et al., 2001). Indeed, the controversy surrounding the use of ECT, especially in the United States, stems primarily from the memory disturbance that is associated with the treatment. Modern ECT procedures, including the use of anaesthesia, oxygenation, and lower currents, however, have greatly reduced the severity of the cognitive side-effects. While there have been a few reports of profound and long-lasting memory disturbance in isolated individuals, the evidence for brain damage after ECT is weak. ECT is a controversial treatment, and some authors have suggested that along with the risks and benefits of treatment, informed consent protocols should include some discussion about this controversy (Reisner, 2003).

Serendipitously, ECT treatment in depressed patients with PD has been found to not only alleviate the depressive symptoms, but to also have beneficial effects on the motor symptoms of PD. Many case studies, open trials, and a few sham-controlled double blind investigations, totalling over 200 patients studied to date, suggest that ECT significantly improves the motor symptoms of PD in over half of these patients, independent of the antidepressant effect (Kennedy et al., 2003; see Table 1.1 for a summary of the literature). The effects are most pronounced in patients with more severe PD or debilitating side effects (e.g. “on-off” phenomenon; Balldin et al., 1981; Andersen et al., 1987). Interestingly, the antiparkinsonian effects are often observed within 3-5 treatments (see Table 1.1), whereas the antidepressant effects take 6-12 treatments to emerge. The amelioration of the motor symptoms of PD by ECT has been reported to last from weeks to months, and can be extended with maintenance treatments (Aarsland et al., 1997; Fall and Granerus, 1999).

Animal studies have been extensively employed to try to understand the mechanism of action of ECT. In animal models, electroconvulsive shock (ECS) has a wide range of effects, from general changes in cerebral blood flow and metabolism to specific changes in neurotransmitter systems, neuroendocrine function, and gene expression (reviewed in Fochtmann, 1994). Most studies to date have been devoted to the study of the mechanism of action of ECT in depression, with intense focus on monoamine neurotransmitter systems in limbic brain regions. Although almost all aspects of neurotransmission seem to be affected by acute ECS treatment, more important are the cumulative effects of repeated treatments, since it takes several treatments to see either an antidepressant or an antiparkinsonian effect in patients.

Electroconvulsive Shock as an Animal Model of ECT

One of the earliest observations after repeated ECS is that it enhances monoamine-mediated behaviours, particularly, those induced by 5-HT- or DA-stimulating drugs (Green et al., 1983a; Green et al., 1983b; Goodwin et al., 1984; Metz and Heal, 1986; and see Table 1.2). Further to those behavioural observations, using in vivo microdialysis, enhancements in the levels of 5-HT and DA in their target brain regions were observed after repeated ECS (Nomikos et al., 1991; Zis et al., 1991; Yoshida et al., 1998a; Yoshida et al., 1998b), as well as increases in the post-synaptic receptors for these receptors, notably the 5-HT2 receptor in the cortex and hippocampus (Kellar et al., 1981; Green et al., 1983a; Stockmeier and Kellar, 1986; Biegon and Israeli, 1987; Pandey et al., 1992; Butler et al., 1993; Burnet et al., 1999) and DA receptors in the dorsal striatum and nucleus accumbens (NAcc; see Table 1.2 for review). For a long time, the changes in 5-HT and DA neurotransmission were used to explain the effectiveness of ECT as an antidepressant treatment, and only recently have the downstream effects of this monoamine activation been investigated.
Table 1.1: Effects of ECT in Parkinson’s disease or parkinsonism:

<table>
<thead>
<tr>
<th>Reference (in chronological order)</th>
<th>Type of study</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lebensohn and Jenkins, 1975</td>
<td>Case study</td>
<td>Amelioration of depression and parkinsonism in two patients with severe PD after 4 ECT</td>
</tr>
<tr>
<td>Dysken et al., 1976</td>
<td>Case study</td>
<td>Alleviation of bradykinesia and rigidity in one patient after 12 ECT (noticeable improvement by 6th ECT)</td>
</tr>
<tr>
<td>Ananth et al., 1979</td>
<td>Case study</td>
<td>Amelioration of drug-induced parkinsonism in one patient after 6 ECT (immediate effects after 1st ECT)</td>
</tr>
<tr>
<td>Baldin et al., 1981</td>
<td>Open trial</td>
<td>Marked improvement of “on-off” symptoms in 5/9 patients, lasting 2-41 weeks</td>
</tr>
<tr>
<td>Andersen et al., 1987</td>
<td>Placebo-controlled, double-blind study</td>
<td>Patients treated with ECT show prolonged “on” phases, decreased motor severity (short-lasting effects); no changes CSF metabolites</td>
</tr>
<tr>
<td>Atre-Vaidya and Jampala, 1988</td>
<td>Case study</td>
<td>Improvement of mania and parkinsonism in one patient after 12 ECT</td>
</tr>
<tr>
<td>Roth et al., 1988</td>
<td>Case study</td>
<td>Improvement of parkinsonism in one patient after 10 ECT (mania and tardive dyskinesia require an extra 7 ECT); anti-depressant and -PD effects last at least 4 months</td>
</tr>
<tr>
<td>Goswami et al., 1989</td>
<td>Longitudinal triphasic</td>
<td>Amelioration of neuroleptic-induced parkinsonism in 9 schizophrenics after 9 ECT</td>
</tr>
<tr>
<td>Douyon et al., 1989</td>
<td>Open trial</td>
<td>Significant improvement in motor function after mean 7 ECT in 7 patients with PD (no depression); significant improvement in 5 patients after only 2 ECT</td>
</tr>
<tr>
<td>Lauterbach and Moore, 1990</td>
<td>Case study</td>
<td>Significant improvement of both parkinsonism and dystonia in one patient after 9 ECT; UPDRS, dystonia, BDI and Ham-D scores fall</td>
</tr>
<tr>
<td>Stern, 1991</td>
<td>Case study</td>
<td>Improvement in PD symptoms after 2nd of 8 ECT; lasts 8 wks</td>
</tr>
<tr>
<td>Zervas and Fink, 1992</td>
<td>Open trial</td>
<td>Improvement in rigidity, tremor, bradykinesia, “on-off” in 4 patients with severe PD after 3-6 ECT; ↓ UPDRS score 20-40%; lasts 4-6 wks; L-DOPA doses reduced</td>
</tr>
<tr>
<td>Friedman and Gordon, 1992</td>
<td>Open trial</td>
<td>3 out of 4 PD patients show markedly improved motor function lasting as long as 8 wks</td>
</tr>
<tr>
<td>Fall et al., 1995</td>
<td>Open trial</td>
<td>16 non-depressed PD patients – all show anti-PD effect lasting days-weeks (50% of patients) or 3-18 months (50% of patients); sig. ↑ CSF HVA and NPY; patients with longest lasting effect show lower CSF 3-MHPG than patients with shorter lasting effect</td>
</tr>
<tr>
<td>Pridmore and Pollard, 1996</td>
<td>Open trial</td>
<td>12 patients with PD – 3 no effect, 5 mild effect (2 wks -30 months), 4 marked effect (10 wks – 35 months)</td>
</tr>
<tr>
<td>Nymeyer and Grossberg, 2002</td>
<td>Case study</td>
<td>Marked improvement after 2nd ECT; L-DOPA must be reduced to eliminate delirium</td>
</tr>
<tr>
<td>Aarsland et al., 1997</td>
<td>Case study</td>
<td>Maintenance ECT prevents relapse in 2 patients with severe PD</td>
</tr>
<tr>
<td>Moellentine et al., 1997</td>
<td>Retrospective study</td>
<td>14/25 PD patients show at least transient improvement in motor function</td>
</tr>
<tr>
<td>Fall and Granerus, 1999</td>
<td>Case study</td>
<td>2 PD patients show even further improvement with maintenance ECT after a course</td>
</tr>
<tr>
<td>Fall et al., 2000</td>
<td>SPECT imaging of DAT</td>
<td>Motor symptoms improve; No change in [123I]-β-CIT uptake after a course of ECT in 6 patients; those with the best uptake improve the most</td>
</tr>
<tr>
<td>Shulman, 2004</td>
<td>Case study</td>
<td>Maintenance ECT for 4 years in late stage PD improves symptoms and mobility</td>
</tr>
</tbody>
</table>

Legend: 3-MHPG = 3-methoxy-4-hydroxyphenyl-glycol; BDI = Beck Depression Inventory; CSF = cerebrospinal fluid; HAM-D = Hamilton Depression Rating Scale; HVA = homovanillic acid; NPY = neuropeptide Y; SPECT = single photon emission computed tomography; UPDRS = Unified Parkinson Disability Rating Scale.
Table 1.2: Changes in DA receptors following repeated ECS:

<table>
<thead>
<tr>
<th>Author</th>
<th>ECS parameters</th>
<th>Technique</th>
<th>Effects of ECS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BEHAVIOUR:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green et al., 1977</td>
<td>6-OHDA</td>
<td>Amphetamine-induced locomotion/ circling</td>
<td>↑ locomotion normal animals, ↑ circling 6-OHDA-treated</td>
</tr>
<tr>
<td></td>
<td>150 V, 1 s</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10/10 d</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>halothane</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wielosz, 1981</td>
<td>150 mA, 0.3 s</td>
<td>Amphetamine-, apomorphine-induced behaviour</td>
<td>↑ spontaneous and drug-induced activity (lasts 5-10 d); no change stereotypical behaviour</td>
</tr>
<tr>
<td></td>
<td>7/7 d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green et al., 1983a</td>
<td>mice</td>
<td>Apomorphine-induced activity</td>
<td>Sig. ↑ apomorphine-induced activity</td>
</tr>
<tr>
<td></td>
<td>90 V, 1 s</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5/10 d</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>halothane</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smith and Sharp, 1997</td>
<td>150 V, 1 s</td>
<td>Agonist-induced behaviour (activity, locomotion,</td>
<td>No change D₁ or D₂ agonists alone; sig. ↑ activity,</td>
</tr>
<tr>
<td></td>
<td>5/10 d</td>
<td>grooming, sniffing, rearing)</td>
<td>locomotion, sniffing w. concomitant admin. D₁ and D₂ agonist or apomorphine (lasts 3 wks)</td>
</tr>
<tr>
<td>Andrade et al., 2002</td>
<td>30 or 120 mC</td>
<td>Apomorphine-induced activity</td>
<td>Activity increases only in rats treated with high dose ECS</td>
</tr>
<tr>
<td></td>
<td>5/5 d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zarrindast et al., 2004</td>
<td>150 mA</td>
<td>Apomorphine-, amphetamine-, SKF 38393- and</td>
<td>↑ apomorphine locomotion @ 7 d, ↑ SKF grooming @ 9 d; no change quinpirole yawning</td>
</tr>
<tr>
<td></td>
<td>8/16 d</td>
<td>quinpirole-induced activity 7-10 d post-ECS</td>
<td></td>
</tr>
<tr>
<td><strong>MICRODIALYSIS:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glue et al., 1990</td>
<td>100 mA, 10 s</td>
<td>Striatal interstitial conc. of DA (during anaesthesia)</td>
<td>Sig. ↑ basal DA</td>
</tr>
<tr>
<td></td>
<td>8/8 d</td>
<td>chloral hydrate (ECS 1 and 8)</td>
<td></td>
</tr>
<tr>
<td>Zis et al., 1991</td>
<td>150 V, 0.75 s</td>
<td>Striatal interstitial conc. of DA and metabolites (freely moving)</td>
<td>Sig. ↑ basal DOPAC and HVA; trend to ↑ DA</td>
</tr>
<tr>
<td></td>
<td>8/15 d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brannan et al., 1993</td>
<td>6-OHDA in SN</td>
<td>Striatal interstitial conc. of DA and metabolites (during anaesthesia)</td>
<td>↑ basal DOPAC, ↓ basal DA in lesioned striatum</td>
</tr>
<tr>
<td></td>
<td>100 V, 2 s</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>chloral hydrate</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8/16 d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yoshida et al., 1998a</td>
<td>125 V, 1.2 s</td>
<td>Striatal interstitial conc. of DA and metabolites (freely moving)</td>
<td>Sig. ↑ basal DOPAC and HVA; trend to ↑ DA</td>
</tr>
<tr>
<td></td>
<td>8/8 d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Author</td>
<td>ECS parameters</td>
<td>Technique</td>
<td>Effects of ECS</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>----------------------</td>
<td>------------------------------------------------</td>
<td>----------------------------------------------------</td>
</tr>
<tr>
<td><strong>RECEPTOR BINDING:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bergstrom and Kellar, 1979a</td>
<td>150 mA, 0.2 s corneal</td>
<td>Binding to striatal homogenates;[^3H]spiroperidol</td>
<td>No change D2 $B_{\text{max}}$ or $K_d$</td>
</tr>
<tr>
<td>Reches et al., 1984)</td>
<td>150 V, 1.5 s 12/28 d</td>
<td>Binding to striatal homogenates;[^3H]spiperone</td>
<td>No effect D2</td>
</tr>
<tr>
<td>Klimek and Nielsen, 1987</td>
<td>100 mA, 1 s 6/11 d</td>
<td>Binding to mesolimbic homogenates;[^3H]SCH 23390</td>
<td>↓ $D_1$ $B_{\text{max}}$ (but n=3 and no proper control group)</td>
</tr>
<tr>
<td>Fochtmann et al., 1989</td>
<td>80 mA, 0.5 s 8/16 d</td>
<td>Binding to sections;[^32P]SCH 23390</td>
<td>29% ↑ $D_1$ binding SN</td>
</tr>
<tr>
<td>Nowak and Zak, 1989</td>
<td>150 mA, 0.5 s 10/10 d</td>
<td>Binding to striatal homogenates;[^3H]SCH 23390</td>
<td>↑ $D_1$ binding</td>
</tr>
<tr>
<td>Barkai et al., 1990</td>
<td>150 V, 1 s 8/8 d</td>
<td>Binding to sections;[^3H]SCH 23390 and spiroperidol</td>
<td>↑ $B_{\text{max}}$ $D_2$ accumbens, amygdala (also ↑ $K_d$ here), claustrum, endopiriform nuc.; ↑ $B_{\text{max}}$ $D_1$ olfactory tubercle, endopiriform, SN</td>
</tr>
<tr>
<td>Sershen et al., 1991</td>
<td>MPTP mice 35 mA, 0.2 s 5/10 d</td>
<td>Binding to striatal homogenates;[^3H]SCH 23390 and[^3H]spiperone</td>
<td>↑ $D_1$ normal and MPTP ↓ $D_2$ MPTP (but no sham group)</td>
</tr>
<tr>
<td>Martin et al., 1995</td>
<td>200 V, 2 s 5/10 d halothane</td>
<td>Binding to striatal homogenates;[^3H]raclopride</td>
<td>No effect D2</td>
</tr>
<tr>
<td>Lammers et al., 2000</td>
<td>85 V, 0.05 s 10/10 d</td>
<td>Binding to sections;[^3H]7-OH-DPAT In situ for $D_3$ mRNA</td>
<td>Sig. ↑ $D_3$ binding and mRNA shell of accumbens (no change $D_1$ or $D_2$ mRNA here)</td>
</tr>
<tr>
<td><strong>NEURONAL ACTIVITY:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chiodo and Antelman, 1980</td>
<td>105 V, 0.7 s 6/6 d</td>
<td>Recording from SNpc neurons; apomorphine-induced responses (0.004 mg/kg – pre-synaptic dose)</td>
<td>↓ sensitivity of autoreceptors ($D_2$)</td>
</tr>
<tr>
<td>Newman and Lerer, 1989</td>
<td>150 V, 1.5 s 10/10 d</td>
<td>Agonist-stimulated adenyl cyclase activity in striatal or “limbic forebrain” homogenates</td>
<td>Upregulation $D_1$, no change $D_2$ both regions</td>
</tr>
<tr>
<td><strong>IN SITU HYBRIDIZATION:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smith et al., 1995</td>
<td>150 V, 1 s 5/10 d halothane</td>
<td>In situ hybridization to $D_1$ and $D_2$</td>
<td>Sig. ↑ $D_1$ and $D_2$ mRNA in the caudal accumbens 4 h, but not 24 h; trend ↑ $D_1$ striatum 4 h but not 24 h</td>
</tr>
</tbody>
</table>

Note: All studies performed on rats and with earclip electrodes and no anaesthesia unless otherwise noted.
One of the primary downstream effects of repeated ECS is the activation of gene transcription. Immediate early genes such as the transcription factors c-fos, c-jun, jun-B, zif268, and NGF-1A all show consistently increased mRNA expression in the hippocampus and cortex after repeated ECS (Cole et al., 1990; Morišobu et al., 1997). In addition, another important transcription factor, cyclic AMP (cAMP) response element-binding protein (CREB) is also activated by repeated ECS treatments (Nibuya et al., 1996). While many genes have cAMP response elements and can be transcriptionally activated by CREB, some of CREB's most common targets include the genes for neurotrophic factors, and CREB is often conceptualized as a survival factor (Walton and Dragunow, 2000). Not surprisingly, there is a growing body of evidence showing that both neurotrophic factor mRNA and protein are elevated in the brain after repeated ECS treatment (reviewed in Table 1.3), and cell growth, particularly in the hippocampus in the form of either mossy fibre sprouting (Gombos et al., 1999; Vaidya et al., 1999; Lamont et al., 2001) or neurogenesis (Madsen et al., 2000; Malberg et al., 2000; Scott et al., 2000), is also a consequence of repeated ECS treatment. Synthesizing the literature, the most widely accepted theory on the mechanism of action of ECT in depression is that repeated ECT treatment enhances 5-HT neurotransmission in the hippocampus, leading to the activation of CREB and changes in gene expression, the induction of trophic factors, in particular brain-derived neurotrophic factor (BDNF), and ultimately increased growth and/or survival of hippocampal neurons (Duman et al., 1997; see “Working Hypothesis” below for more detail).

Table 1.3: Brief review of the literature on the effects of repeated ECS on the neurotrophic factors BDNF, FGF-2, and GDNF

<table>
<thead>
<tr>
<th>Growth Factor</th>
<th>Change</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDNF</td>
<td>↑ BDNF protein and mRNA hippocampus, protein frontal cortex</td>
<td>Jacobsen and Mork, 2004</td>
</tr>
<tr>
<td></td>
<td>↑ BDNF protein entorhinal, parietal, frontal cortex, septum, hippocampus, striatum</td>
<td>Altar et al., 2003</td>
</tr>
<tr>
<td></td>
<td>↑ BDNF protein hippocampus, occipital cortex, striatum</td>
<td>Angelucci et al., 2002</td>
</tr>
<tr>
<td></td>
<td>↑ BDNF mRNA dentate gyrus and piriform cortex</td>
<td>Zetterstrom et al., 1998</td>
</tr>
<tr>
<td></td>
<td>↑ BDNF and trkB mRNA dentate gyrus, CA3, piriform cortex; ↑ BDNF mRNA CA1</td>
<td>Nibuya et al., 1995</td>
</tr>
<tr>
<td>FGF-2</td>
<td>↑ FGF-2 protein entorhinal, frontal cortex</td>
<td>Gwinn et al., 2002</td>
</tr>
<tr>
<td></td>
<td>↑ FGF-2 mRNA entorhinal, frontal cortex</td>
<td>Kondratyev et al., 2002</td>
</tr>
<tr>
<td></td>
<td>↑ FGF-2 mRNA entorhinal cortex, olfactory bulb, hippocampus</td>
<td>Follesa et al., 1994</td>
</tr>
<tr>
<td>GDNF</td>
<td>↓ GDNF protein hippocampus, striatum</td>
<td>Angelucci et al., 2002</td>
</tr>
<tr>
<td></td>
<td>↑ GFRα-1 and -2 mRNA dentate gyrus, GFRα-1 CA3, reticular thalamic nucleus, GFRα-2 parietal cortex (6 h); no effect on GDNF or RET mRNA</td>
<td>Chen et al., 2001</td>
</tr>
</tbody>
</table>
Overview and Objectives:

The overall objective of this body of work was to provide some insight into the question, “What is the mechanism of action of ECT in PD?”. In order to address that question, however, many steps were taken along the way, and the experimental model, the tools used, and the hypotheses all evolved as our knowledge and experience increased. This thesis is a chronological description of that journey, and each of the central chapters represents a manuscript that has either been published, or has been submitted for publication, and each is self-contained with Introduction, Materials and Methods, Results, Discussion, and Reference sections. Chapters 2 – 4 and Appendices A and B outline the early pilot and validation studies, while Chapters 5 and 6 address the primary working hypothesis. To mitigate the abruptness of the transitions between the chapters, a preamble has been added to each of the central chapters, to help link the ideas from one chapter to the next. The final chapter, Chapter 7, General Discussion, is meant as just that – a general discussion of the body of work, its impact, significance, and limitations. More specific discussion of the experimental results can be found within each of the central chapters.

One of the unique aspects of this body of work is that it bridges the fields of Neurology and Psychiatry; Neurology in its examination of the neurodegenerative disease, PD, and Psychiatry in its examination of ECT, a widely used psychiatric treatment. Similarly, there is also a split focus on two different (yet interrelated) neurochemical systems, the DA and 5-HT systems, each associated with the Neurological and Psychiatric aspects of this thesis, respectively. Biological systems, however, are never black and white, and this is also true of the topic being discussed here. Crossover, interaction, parallelism, and synergism all occur between Neurology and Psychiatry, the 5-HT and DA systems. For instance, there are psychiatric aspects of PD, including mood disorders and antiparkinsonian drug-induced psychosis. Interactions between the 5-HT and DA systems have only recently been recognized, but are now being targeted, for example by modulating the 5-HT system to treat the symptoms of PD (Nicholson and Brotchie, 2002). Likewise, there is a great deal in common between the DA and 5-HT systems, not only in their chemical make-up (both neurotransmitters are monoamines derived from precursor amino acids), or their neuroanatomy (both make widespread projections primarily from nuclei within the brainstem), but also in their function (both play a role in modulating mood and attention).

A great deal of attention has been paid to the role of 5-HT in the area of depression. There is a large body of evidence suggesting that 5-HT is depleted in limbic brain regions such as the frontal cortex and hippocampus in major depressive disorder, and the aim of most antidepressant treatments, therefore, is to enhance 5-HT neurotransmission. Once ECT was recognized as an effective treatment for depression, much of the investigation into its mechanism of action also focused on the 5-HT system. Because so much is known about the 5-HT system with respect to mood disorders and their treatment with either antidepressant drugs or ECT, we used the 5-HT system as a frame of reference in the early stages of this project, in evaluating the most appropriate animal models to employ, to develop the tools to address our questions, and even to develop our working hypothesis.
Model and Tool Development:

In order to evaluate the effects of ECS in parkinsonism, it was necessary to first carefully evaluate the animal models being employed. We initially investigated the possibility of performing the bulk of our investigations into ECS-induced changes in monoamine neurotransmission in a non-human primate model, as this species is ideally suited for longitudinal follow up of neurochemical changes by positron emission tomography (PET) and we already had extensive experience with both the model and the technique. As mentioned above, because ECS is known to affect 5-HT neurotransmission in rodents, we decided to look not only at the DA system, but also at the 5-HT system in our non-human primate subjects. While the effects of repeated ECS on 5-HT2 receptor binding were clear (Chapter 2), these initial studies also helped us to identify the limitations of this particular model and technique. Recognizing that neither a non-human primate model, nor the use of PET as our research tool would allow us to fully dissect the effects of ECS in an appropriate number of subjects or a reasonable time frame, we opted to switch to a rodent model and adopted a variety of different techniques.

In moving from the non-human primate to the rodent, it was necessary to develop a technique to use the same radiopharmaceuticals used in vivo with PET imaging to quantify changes in monoamine receptors in vitro in tissue slices. As such, a major project of this doctoral research was the development of a quantitative autoradiographic technique using PET tracers (Chapter 3), a technique which was later applied at several stages to help address the main research questions. In this chapter, we again took advantage of the known effects of antidepressant treatments on the 5-HT system and chronically treated rats with the antidepressant drug desipramine (DMI), a pharmacological intervention that robustly downregulates cortical 5-HT2 receptors, in order to validate our technique.

One of the glaring discrepancies in the literature regarding the effects of antidepressant treatments on the 5-HT system is that the 5-HT2 receptor is typically downregulated by antidepressant drug treatment in humans and rodents (and, as we have shown, by ECS in non-human primates), but that ECS in rodents has been repeatedly shown to upregulate this receptor. Before embarking on a series of studies using rodent ECS as our model, then, it was important to investigate this discrepancy further. We hypothesized that the discrepancy may arise from a technical difference between the administration of ECT to humans compared to ECS to rodents, the fact that humans are always anaesthetized, whereas rodents typically are not. Because we wanted to model the clinical situation as closely as possible, we decided to use anaesthesia when treating our rodent subjects with ECS. We therefore performed a small pilot study investigating the use of anaesthesia for ECS in rats, and, after selecting ketamine as our anaesthetic of choice for all future studies, also investigated the effects of ECS under ketamine on frontal cortex 5-HT2 receptor binding in rats (Appendix A).

Over the last several years, our laboratory has also focused on developing a battery of non-pharmacological behavioural tests to examine motor behaviour in the 6-OHDA-lesion model. In doing so, it was important to show that the tests were sensitive to the overt behavioural changes that occur in the 6-OHDA model, and that there is a good correspondence between the neurochemical and behavioural changes. Two behavioural tests were employed in addressing the effects of ECS on DA-mediated motor behaviours, the Cylinder Test, and the Tapered/Ledged Beam-Walking Test, and the results of a thorough evaluation of the latter can be found in Chapter 4.

Knowing the activity-dependent nature of BDNF expression, its induction by repeated ECS treatment in rodents (Table 1.3), and the potential of this neurotrophic factor to ameliorate DA deficits in models of PD, we also performed an early pilot study investigating the effects of repeated ECS treatment on BDNF mRNA expression in 6-OHDA-lesioned rats (Appendix B).
Our inability to detect BDNF mRNA in our primary region of interest, the striatum, led us to examine protein levels directly for our later studies examining neurotrophic factors. After performing these initial pilot and validation studies, allowing us to select the most appropriate animal model and to develop the necessary tools, we were ready to start investigating our hypotheses regarding the mechanism of action of ECT in PD.

**Hypotheses:**

To understand the mechanism of action of ECT in PD, we must take into account the nature of the stimulation being applied, and the anatomy of the system that it is being applied to. In ECT, electrical current is applied to the surface of the brain, directly stimulating the cerebral cortex, but only indirectly stimulating subcortical structures such as the hippocampus and striatum by activating corticofugal projections. The striatum is uniquely positioned to integrate signals coming into the basal ganglia from the cortex and SN, and to transmit the integrated signal out of the basal ganglia via either the direct or indirect pathways to modulate motor output. We hypothesize that in the parkinsonian brain, ECT enhances striatal DA function by indirectly stimulating the striatum, leading to changes in the expression of DA receptors, trophic factors, and ultimately influencing motor output.

**Working Hypothesis:**

Our hypotheses regarding the specific cellular and molecular effects of ECS in the parkinsonian striatum is simply a transposition of a theory provided by Duman and colleagues (1997) to explain the effects of ECT in the hippocampus in depression. Based on the known effects of ECS on monoamine neurotransmission, they investigated the downstream effects. Through an elegant series of experiments, they have provided support for their hypothesis that the common target of antidepressant drugs and ECT is the transcription factor CREB, and that CREB is responsible for increased neurotrophic factor content and neural plasticity in the hippocampus after ECS. Ultimately, they have shown that mRNA for BDNF and its receptor, trkB, are upregulated in the hippocampus after a course of ECS (Nibuya et al., 1995), that ECS induces both sprouting and neurogenesis in the hippocampus (Vaidya et al., 1999; Malberg et al., 2000), and that the neurogenesis results from activation of CREB (Nakagawa et al., 2002).

Our hypothesis is that ECS has similar effects in the lesioned striatum as it has in the hippocampus, and we have developed a working hypothesis based on this theory, in an attempt to explain the primary observation that ECT treatment improves the motor symptoms of PD (Figure 1.1). By bringing together five observations on the effects of ECS in the normal brain, and placing them in the context of the striatum, we have developed a framework for our investigations. (1) Repeated ECS increases basal striatal DA release (Zis et al., 1991; Yoshida et al., 1998a), which could increase the activation of D1 receptors. D1 receptors are positively coupled to protein kinase A (PKA), and one of PKA's main downstream targets is CREB. (2) CREB initiates the transcription of many genes, including the trophic factors BDNF and glial cell line-derived neurotrophic factor (GDNF; Shieh et al., 1998; Woodbury et al., 1998). The neurotrophic factors can have several downstream effects, including (3) enhanced growth and survival of DA neurons, and, in particular for BDNF, (4) the induction of the D3 (Guillin et al., 2001) and perhaps even the D1 (Do and Kuzhikandathil, 2005) receptor. In addition, repeated ECS treatment could (5) increase striatal neurotrophic factor release (e.g. BDNF is released from corticostriatal projections (Altar et al., 1997), which could lead to increased SN neurotrophic factor concentration [BDNF, GDNF and basic fibroblast growth factor (FGF-2) are all transported retrogradely from the striatum to the SN (Mufson et al., 1999)], which, again, can influence DA neuron survival and striatal DA receptor expression. Improved motor performance can occur as a result of any one of these effects.
IMPROVED MOTOR PERFORMANCE

Legend:
○ = neurotrophic factor protein
○ = dopamine

Adapted from Duman et al, 1997.
Figure 1.1: Working hypothesis to explain the mechanism of action of ECT in PD. ECS has several potential effects in the striatum that can influence motor output. (1) Repeated ECS increases basal striatal DA release, which could increase the activation of $D_1$ receptors, which can lead to the activation of the transcription factor CREB. (2) CREB can initiate the transcription of many genes, including those for neurotrophic factors. The neurotrophic factors can have several downstream effects, (3) including enhanced growth and survival of DA neurons, and, in particular for BDNF, (4) the induction of the $D_3$ receptor. In addition, repeated ECS treatment could increase neurotrophic factor release from corticostriatal projections (5), which, again, can influence (3) and (4). Improved motor performance can occur as a result of any one of these 5 effects.
We chose to examine three specific aspects of the pathway to provide support for our working hypothesis. The overt effect of ECT in PD is a behavioural improvement, so our first goal was to examine motor behaviour after repeated ECS treatment in 6-hydroxydopamine (6-OHDA)-lesioned rats. Secondly, one of the most robust effects of ECS is upregulation of postsynaptic monoamine receptors, and we specifically chose to investigate the D_1 and D_3 receptors because they are upregulated by ECS in normal rats and because of their role in motor output. Finally, since neurotrophic factors have great potential as therapeutics for PD and since repeated ECS in rats enhances neurotrophic factors, we chose to investigate the effects of repeated ECS on trophic factor concentration in 6-OHDA-lesioned rats.

**Hypothesis 1): Repeated ECS improves motor performance in 6-OHDA-lesioned rats**

The most robust observation of the effects of ECT on PD patients is a fairly immediate and long-lasting improvement in their motor symptoms. Indeed, if ECT is to be promoted as an adjunct therapeutic intervention for PD, it must improve the motor deficits experienced by patients. Very few studies have examined motor behaviours after ECS treatment in rodents, and those have only looked at drug-induced behaviours in normal (Wielosz, 1981; Green et al., 1983b; Smith and Sharp, 1997) or unilateral 6-OHDA-lesioned rats (Green et al., 1977; see Table 1.2 for review). We hypothesize that non-pharmacological motor behaviour of unilateral 6-OHDA-lesioned rats will also improve after a course of ECS treatment. To test this hypothesis, we will employ two behavioural tests of DA-dependent motor function. The Forelimb Use Asymmetry Test (Cylinder Test) is a test of forelimb function, and takes advantage of the natural exploratory behaviours of rodents. The Cylinder Test is becoming widely adopted as a sensitive and simple test for evaluating 6-OHDA-induced forelimb deficits and improvement of those deficits after various interventions (Shi et al., 2004; Dowd et al., 2005; Cepeda et al., 2006). The Tapered/Ledged Beam-Walking Test (TB test) is an adaptation of simpler beam-walking tasks, again capitalizing on the natural behaviours of the rat, and taking into account the animals’ propensity to compensate for lesion-induced changes in their motor function (Schallert and Woodlee, 2005; also see Chapter 4). We hypothesize that both forelimb and hindlimb function, as measured by changes in the Cylinder and TB tests respectively, will be improved by repeated ECS treatment. This hypothesis is addressed in Chapter 5.

**Hypothesis 2): Repeated ECS upregulates striatal D_1 and D_3 receptors**

Early studies of the effects of ECS on the brain focused on neurotransmitters and their receptors, mainly because the known site of action of antidepressant drugs was at that level. In particular, there is a vast literature providing evidence that repeated ECS has effects on DA receptors (Table 1.2). One of the more replicated findings is that repeated ECS increases the locomotor response to DA agonists and releasing agents (apomorphine and amphetamine), suggesting post-synaptic receptor upregulation (Green et al., 1977; Wielosz, 1981; Green et al., 1983a; Smith and Sharp, 1997). Indeed, the motor activating effect of repeated ECS cannot be attributed to either the D_1- or D_2-like receptors alone, but appears to result from stimulation of both receptor subtypes (Smith and Sharp, 1997).

Examination of the effects of repeated ECS treatment on DA receptors at a cellular level indicates that the changes are widespread, and are region and subtype specific. Using both homogenate and autoradiographic receptor binding techniques, upregulation of D_1 receptors is a common finding in the normal SN and striatum after a course of ECS (Fochtmann et al., 1989; Nowak and Zak, 1989; Barkai et al., 1990), while D_2 receptors are unchanged in the striatum (Bergstrom and Kellar, 1979b; Reches et al., 1984; Sershen et al., 1991; Martin et al., 1995), and D_2 and D_3 receptors have only been reported to be upregulated in the NAcc (Barkai et al., 1990; Lammers et al., 2000). In addition, one study of the effects of ECS in DA-depleted animals,
shows increased binding to striatal D₁ receptors, but decreased binding to D₂ receptors (Sershen et al., 1991).

The D₃ receptor is most abundant in the NAcc, but is expressed at very low levels in the dorsal striatum and the rest of the brain under normal circumstances (Diaz et al., 1995). It can be induced in the striatum by the application of very specific stimuli, however, including chronic L-DOPA treatment (Bordet et al., 1997; van Kampen and Stoessl, 2003), and, more importantly for this body of work, by BDNF (Guillin et al., 2001). Recent evidence suggests that the D₁ receptor may also be induced by BDNF (Do and Kuzhikandathil, 2005). Interestingly, D₁ and D₃ receptors are thought to be co-expressed by the same neurons within the ventral striatum (Ridray et al., 1998).

Based on the literature of the effects of ECS on DA receptors in the normal brain, we expect to see increased binding to D₁ and D₃ receptors, and no changes in D₂ binding in the mesostriatal DA system of unilateral 6-OHDA-lesioned animals after a course of ECS. This hypothesis is addressed in Chapter 5.

**Hypothesis 3:** Repeated ECS induces the trophic factors BDNF, GDNF and/or FGF-2 in the striatum of 6-OHDA-lesioned rats

Degeneration of nigrostriatal DA neurons is the hallmark pathology in PD. Treatments that can stop degeneration and protect the remaining neurons and/or induce the growth of new, functional neurons are the ultimate treatments of choice for all neurodegenerative disorders. In PD, therapeutic treatments that protect and/or replace nigrostriatal DA neurons have been difficult to develop and have mainly focused on cell transplant surgery, which carries with it some risk to the patient and the potential for side effects. Treatments that can protect or regenerate DAergic neurons without the administration of exogenous factors are therefore favourable.

Neurotrophic factors are large proteins that play a role in the development and growth of the nervous system, and have neuroprotective effects in the adult brain. In recent years, various techniques have identified neurotrophins as one of the most consistent and robust targets of ECS. Gene microarray studies have shown that, while the expression of many genes is altered by ECS, some specific pathways are affected. In particular, in the hippocampus and cerebral cortex, growth-promoting genes appear to be enhanced by ECS, including genes for angiogenesis, neurogenesis, immediate-early genes, brain BDNF and its downstream signalling molecules, and FGF-2 (Newton et al., 2003; Altar et al., 2004). We hypothesize that in the parkinsonian brain, three specific neurotrophins, BDNF, FGF-2 and GDNF may play a role in the positive effects of ECS for several reasons: 1) all three neurotrophins are reduced in the brains of PD patients; 2) they have positive effects in animal models of PD and in PD patients; and 3) the proteins and/or their receptors are upregulated by repeated ECS treatment (Table 1.3).

The classical signalling mechanism of neurotrophic factors is via retrograde transport from the axon terminal, where they are taken up and packaged for transport back to the cell body, where they exert positive effects on cell growth and survival. Under normal circumstances, BDNF, GDNF, and FGF-2 are all transported retrogradely from the striatum to the DA cell bodies of the SN (Mufson et al., 1994; Tomac et al., 1995b; Mufson et al., 1996; Mufson et al., 1999). This major source of trophic support is jeopardized in PD, and BDNF, GDNF, and FGF-2 protein are all decreased in the SN of PD patients (Tooyama et al., 1993; Mogi et al., 1999; Chauhan et al., 2001).

In animal models of PD, administration of BDNF, GDNF, or FGF-2 protects against striatal DA loss, loss of tyrosine hydroxylase (TH; the rate-limiting enzyme in DA production) positive cell bodies in the SN, and motor dysfunction induced by the neurotoxins 6-OHDA and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; Otto and Unsicker, 1990; Shults et al.,
1995; Tomac et al., 1995a; Vaidya et al., 1997; Bowenkamp et al., 1997; Shults et al., 2000; Sun et al., 2005). To be effective treatments for PD, however, trophic factors must also be able to restore the function of DA neurons, because by the time a diagnosis of PD has been made, the patient has generally lost approximately half of their nigrostriatal DA neurons and 80% of striatal DA (Bernheimer et al., 1973). Both GDNF and FGF-2 have been shown to have restorative effects after DA lesions in animal models. Intracerebroventricular (ICV) administration of FGF-2 in MPTP-treated monkeys or mice improves motor performance, striatal DA function, and the number of TH+ cell bodies in the SN (Chadi et al., 1993; Fontan et al., 2002). Intrastriatal or ICV infusion of GDNF increases the number of nigral DA neurons and striatal DA content, and improves motor function in 6-OHDA-lesioned rats and MPTP-treated monkeys (Aoi et al., 2000; Costa et al., 2001; Kirik et al., 2001; Grondin et al., 2002). Gene delivery of GDNF using viral vectors is also an effective means of enhancing DA function after lesioning in rats and primates (Lapchak et al., 1997; Kordower et al., 2000; Wang et al., 2002; Dowd et al., 2005).

The positive effects of ECT on the motor symptoms of PD may be at least partially due to its induction of endogenous trophic factors in the nigrostriatal pathway. In normal animals, ECS increases the concentration of both mRNA and protein for FGF-2, BDNF and GDNF in many brain regions (Table 1.3). BDNF shows robust increases, particularly in the hippocampus, cortical regions, and the striatum (Nibuya et al., 1995; Zetterstrom et al., 1998; Angelucci et al., 2002; Altar et al., 2003; Jacobsen and Mork, 2004). Although BDNF has been the most thoroughly investigated neurotrophin with respect to the effects of ECS, mRNA and protein expression of FGF-2 have also been shown to increase in the cortex and hippocampus after minimal ECS (Follesa et al., 1994; Gwinn et al., 2002; Kondratyev et al., 2002). In contrast, while ECS treatment increases mRNA for the GDNF receptors GFRα-1 and GFRα-2 in the hippocampus and parietal cortex, GDNF mRNA appears to be unaffected by ECS treatment (Chen et al., 2001). So far, only one study has looked at GDNF protein after ECS (Angelucci et al., 2002), and it showed significant decreases in both the striatum and hippocampus.

Because BDNF, GDNF and FGF-2 are lost in the SN of PD patients, have therapeutic effects in PD and its animal models, and because the concentrations of all three proteins in the brain is influenced by repeated ECS treatment (Table 1.3), we hypothesize that the striatal levels of BDNF, FGF-2, and perhaps to a lesser extent, GDNF, will be increased by repeated ECS treatment in unilaterally 6-OHDA-lesioned rats. This hypothesis is addressed in Chapter 6.
References:


Dysken M, Evans HM, Chan CH, Davis JM (1976) Improvement of depression and parkinsonism during ECT. Neuropsychobiol 2: 81-86.


Metz A, Heal DJ (1986) In mice repeated administration of electroconvulsive shock or desmethylimipramine produces rapid alterations in 5-HT2-mediated head-twitch responses and cortical 5-HT2 receptor number. Eur J Pharmacol 126: 159-162.


Smith SE, Sharp T (1997) Evidence that the enhancement of dopamine function by repeated electroconvulsive shock requires concomitant activation of D1-like and D2-like dopamine receptors. Psychopharmacology 133: 77-84.


Chapter 2

ELECTROCONVULSIVE SHOCK DECREASES BINDING TO 5-HT$_2$ RECEPTORS IN NON-HUMAN PRIMATES: AN IN VIVO PET STUDY WITH [¹⁸F]SETOPERONE

Preamble:
We performed our preliminary investigations into the effects of repeated ECS treatment on brain neurochemistry using PET imaging in non-human primates. The primary advantage of PET over other techniques is that it allows the investigation of neurochemical changes in vivo in the same animals over time, and we took advantage of that characteristic in this chapter. Non-human primates offer a unique opportunity to perform pre-clinical investigations of potential therapeutic interventions, such as the use of ECT for PD, because their physiology, behaviour, and, in particular, their neuroanatomy, are similar to that of humans. There are also disadvantages to using non-human primates compared to rodents, including the ethical requirement of using as few subjects as possible, the financial costs of acquiring and maintaining these animals, and a higher degree of variability in behaviour and neurochemistry. Although we were mainly interested in the effects of repeated ECS treatment on the DA system, we were also interested in examining the 5-HT system, since this system has been the most thoroughly investigated after both ECT treatment in humans and ECS treatment in rodents, in an attempt to understand the mechanism of action of ECT in depression. Whenever the availability of the cyclotron and PET scanner allowed, we also performed a few pilot PET studies using a tracer for D$_1$ receptors. This data has not been published, as the number of subjects was small, but our analysis showed a trend towards increased D$_1$ receptor binding, as expected from the rodent literature. These pilot PET studies did, however, demonstrate to us that, although the primate model would be the best to perform our investigations, the technical difficulties associated with primate access, ECT delivery and PET scheduling severely compromised our ability to perform studies with a sufficient number of subjects in a reasonable time frame. Thus, considering all these facts, we opted to switch to a rodent model, allowing for more invasive studies and an easier dissection of the effects of ECS, and the ability to examine greater numbers of subjects.

Introduction:

Electroconvulsive therapy (ECT) is widely and effectively used to treat psychiatric disorders, and is particularly indicated in unipolar major depression. In contravention to the recommendations of a recent American Psychiatric Association Task Force on ECT (2001), it is typically relegated to the position of last resort, only introduced when adequate trials of antidepressant drugs fail to improve the patient’s mood disorder. However, it is increasingly being recognized as the most effective antidepressant treatment available to patients (Kho et al., 2003; UK ECT Review Group 2003; Pagnin et al., 2004). Recent research has advanced our understanding of the molecular and cellular bases of the mechanism of action of effective antidepressant treatments (for review see Vaidya and Duman 2001; Donati and Rasenick 2003), but many questions remain unanswered. Both pharmacological treatments and ECT require repeated exposure over 3-4 weeks to obtain an antidepressant effect, suggesting that acute biochemical changes cannot entirely explain the long-term effect, and yet few longitudinal studies have been performed to evaluate the time course and persistence of these effects.

A version of this chapter has been published. Strome EM, Clark CM, Zis AP, Doudet DJ (2005) Electroconvulsive shock decreases binding to 5-HT$_2$ receptors in non-human primates: an in vivo PET study with [¹⁸F]setoperone. Biological Psychiatry 57(9): 1004-1010.
In view of the common mode of action of most effective antidepressant drugs [enhancement of synaptic serotonin (5-HT)], attenuation of 5-HT neurotransmission, particularly within cortical regions, is thought to play a major role in the pathophysiology of depression. Therefore, normalizing serotonergic activity may represent a crucial aspect of the mechanism of action of both antidepressant drugs and ECT. Evidence for changes in 5-HT neurotransmission in depression, however, is mainly indirect, through observation of changes in 5-HT receptor binding. While the majority of post mortem studies of depressed patients or suicide victims show elevated 5-HT2 receptor density in the frontal cortex (Yates et al., 1990; Hrdina et al., 1993), in vivo positron emission tomography (PET) studies of unmedicated depressed patients show elevations (Meyer et al., 2003), reductions (Attar-Levy et al., 1999; Yatham et al., 2000; Messa et al., 2003), or no evidence for changes in cortical 5-HT2 binding (Meyer et al., 1999). The variability of results obtained within and among PET and post-mortem studies may arise from heterogeneity within either the diagnosis of the patients studied, or their history of antidepressant treatment (reviewed in Stockmeier 2003). In contrast, successful treatment with antidepressant drugs, ranging from tricyclics to selective serotonin reuptake inhibitors (SSRIs) to mixed SSRI/5-HT2 antagonists, consistently decreases cortical 5-HT2 binding back to control levels (Attar-Levy et al., 1999; Yatham et al., 1999; Mischoulon et al., 2002).

The effects of ECT on 5-HT2 receptors have not been determined in humans, but have been studied extensively in a rodent model. In fact, one of the most consistent findings after chronic treatment of rodents with electroconvulsive shocks (ECS) is an increase in cortical 5-HT2 receptor binding and mRNA expression (Bergstrom and Kellar 1979; Biegon and Israeli 1987; Burnet et al., 1999). As far as antidepressant treatment effects on 5-HT2 receptors goes, this upregulation in rodent ECS is unexpected, based on the wide range of pharmacological antidepressants that decrease cortical 5-HT2 receptor binding in both humans and rodents (Peroutka and Snyder 1990; Yatham et al., 1999; Mischoulon et al., 2002; Strome et al., 2005). Species differences in cortical circuitry may explain the unexpected upregulation of 5-HT2 receptors after ECS in rodents, and the effects of ECT on 5-HT2 receptors may therefore be more accurately modelled in a species that is phylogenetically closer to humans, such as non-human primates.

There are several families of 5-HT receptors, with the 5-HT2 family currently divided into 5-HT2A, 5-HT2B and 5-HT2C subtypes. While the 5-HT2 family is difficult to differentiate pharmacologically, the distribution of these receptors is specific, with the 5-HT2A and 5-HT2C subtypes predominating cortically, and throughout the brain, while the 5-HT2B receptor has a limited central distribution (reviewed in Hoyer et al., 2002). Setoperone is an antagonist with high affinity (Kd = 0.7 nM; Maziere et al., 1988) and specificity for serotonin 5-HT2 receptors, with higher affinity for the 2A than the 2C subtype. Although setoperone also binds to dopamine D2 receptors, its affinity is 50 times less than for 5-HT2 receptors (Leysen and Gommeren 1986). In the human and non-human primate brain, [18F]setoperone shows marked retention in the cortex and striatum, and complete washout in the cerebellum (Blin et al., 1988; 1990). The tracer [18F]setoperone is an excellent ligand to image cortical 5-HT2 receptors in vivo, and has been used successfully by many groups for that purpose (Yatham et al., 1999, 2000; Meyer et al., 1999, 2003; Mischoulon et al., 2002).

We studied the effects of a clinical course of ECS in rhesus monkeys (Macaca mulatta) in vivo using PET and [18F]setoperone, to examine the extent and persistence of changes to cortical 5-HT2 receptor binding induced by electroconvulsive stimulation in a primate species. PET is a powerful tool for studying changes in the biochemistry of discrete brain regions longitudinally, and non-human primates provide an excellent model to investigate the mechanism of action of ECT because their neuroanatomy is similar to humans, and they display complex social behaviour.
Materials and Methods:

**Subjects**

Seven adult male rhesus monkeys (aged 16-22 years, 8.5-17.0 kg) were the subjects of this experiment. The animals were housed in dyads in large (4' x 4' x 5') chain-link enclosures with tree-trunk perches, foraging, and other toys available, and had view of the entire colony room (with 4-5 other pairs of animals housed similarly). Older, fully mature animals were chosen to mimic the demographic of the human population most often receiving ECT, middle-aged and elderly patients. Each animal was healthy and had not been involved in pharmacological studies or received any drugs, apart from those necessary for veterinary care (mainly antibiotics and anaesthetics) and anaesthetics (ketamine and isoflurane for the performance of PET studies) for over 5 years.

**ECS Procedure**

For the ECS session, the animal was anaesthetized with ketamine HCl (10 mg/kg i.m.), and given atropine (0.05 mg/kg i.m.) to reduce secretions, and succinylcholine (1 mg/kg i.m.) for muscle relaxation. The animal was intubated to maintain airway patency, and positioned supine on a procedure table. A clinical ECT device (Thymatron, Somatics Inc., Lake Bluff, IL) set to a frequency of 70 Hz, a pulse width of 0.5 msec and a 0.9 A current was used to administer ECS. The electrodes were placed bilaterally on the temples by an investigator skilled in the administration of ECT to both humans and animals (APZ). Bilateral electrode placement was adopted because this configuration is used clinically to obtain maximal efficacy, and because the size and shape of the monkey’s head limits the separation of the electrodes in a unilateral configuration, and shorter inter-electrode distances are associated with decreased efficacy in humans (Ottoson 1991). Seizure length was monitored through visual observation of the motor seizure and timing with a stopwatch. Seizure threshold (electrical charge in milliCoulomb necessary to induce a generalized seizure lasting at least 25 sec) was determined during the first treatment using the titration method (Sackeim et al., 1987a). As in human ECT protocols, the applied current was 1.5 times the seizure threshold for the second treatment, and was increased as required to continue to elicit motor seizures of approximately 20 s duration as the course progressed, using the stimulus duration to adjust the dose of current. After the procedure, the animal was allowed to awaken spontaneously. No negative side effects of repeated anaesthesia or ECS were observed in any of the animals. Each animal was treated twice per week for 3 weeks, for a total of 6 treatments.

**PET Procedure**

The radiosynthesis of \([^{18}F]setoperone\) was as previously described (Adam et al., 1997). PET scans were performed on each monkey prior to the first ECS treatment, and 24 hours, 1 week and 4-6 weeks after the last ECS. Five of the seven animals also had a PET scan 24 h after the second or third ECS treatment. Preparation of the animal for scanning has been described in detail elsewhere (Doudet et al., 1995; Strome et al., 2002). Briefly, the animal was immobilized with ketamine HCl (10 mg/kg i.m.), given atropine (0.05 mg/kg i.m.), was intubated, and had a venous line inserted. After initial anaesthesia with a low dose of sodium pentobarbital (7-10 mg/kg i.v.) for transport to the PET suite, the animal was maintained under light anaesthesia with isoflurane gas (0.5 -1% in O2) for the remainder of the study. The animals underwent a \([^{11}C]raclopride\) scan prior to every \([^{18}F]setoperone\) scan to assess the state of striatal dopamine D2 receptors. Thus, at least 4 hours elapsed between initiation of barbiturate anaesthesia and injection of \([^{18}F]setoperone\).
The animal was positioned prone in a stereotaxic frame, allowing the acquisition of 31 coronal slices of the head and brain. The PET scans were obtained using a Siemens ECAT 953-31B tomograph, with in-plane and axial resolution of 5-6 mm full-width at half-maximum. A transmission scan was performed for attenuation correction. The $[^{18}\text{F}]$setoperone dose (5 mCi in 10 ml saline) was administered i.v. over 1 min with a Harvard pump (Harvard Apparatus, Holliston, MA). Scanning in 2-D mode started at injection and lasted 120 min.

All animal procedures were approved by the Committee on Animal Care at the University of British Columbia.

**Data Analysis**

Data obtained from 60 to 120 min after tracer injection were summed, and from the 60-120 min activity images, we determined the ratio of the radioactivity concentration in areas of specific $[^{18}\text{F}]$setoperone accumulation, compared to one area of non-specific binding, the cerebellum (which has very few 5-HT$_2$ receptors; Pazos et al., 1987). We chose to analyze the 60-120' post injection data because region:cerebellum $[^{18}\text{F}]$setoperone binding ratios increase in the first hour after injection, but remain stable for the second hour (Blin et al., 1990), and because the region:cerebellum ratio obtained during this time period is strongly correlated with the binding potential obtained from kinetic tracer modelling (Petit-Taboue et al., 1996). Furthermore, the test-retest reliability of the ratio method is very high (5-7%) and has been used to detect within subjects differences of 10% or more, and between groups differences of at least 25% in human subjects (Kapur et al., 1997). Regions of interest (ROI) analysis was performed by an investigator experienced in the analysis of PET data (DJD). For each animal, a well-characterized template (Doudet et al., 1995; Strome et al., 2002) consisting of multiple small ROIs was placed on 19 consecutive brain slices in the baseline (pre-ECS) scan. The position of the ROIs was adjusted based on the individual animal’s neuroanatomy and the radioactivity within a region. The ROIs placed on a single brain area, but on several contiguous slices were averaged to give a measure of 5-HT$_2$ binding. The binding data were then converted to the region:cerebellum ratio for statistical analysis, and are presented as that ratio minus one (equivalent to the binding potential) for graphical representation. After matching and alignment to the baseline scan, the ROI template for each animal at baseline was then re-placed on their subsequent four (or five) scans (mid-ECS, 24 hours, 1 week and 4-6 weeks post-ECS), and regional binding potentials determined as above.

**Statistical Analysis**

Analysis of seizure characteristics was performed with GraphPad Prism version 3.00 for Windows (San Diego, CA). The effects of ECS treatment on 5-HT$_2$ binding throughout the cortex over time were investigated by two-way repeated measures analysis of variance (ANOVA), with TIME and REGION the factors, using StatSoft Statistica v5.1 (Tulsa, OK) software. Significant main effects in the ANOVA were investigated further with post-hoc testing (Tukey test).

To examine the pattern that the changes in 5-HT$_2$ binding followed over time, the binding data were further analyzed using the method of orthogonal polynomials (Hays 1988) using SPSS v12.0 (Chicago, IL) software. Briefly, this involves performing a one-way analysis of variance with TIME as the within term, and then partitioning the TIME effect into three orthogonal polynomials to test for linear, quadratic and cubic effects. With respect to the experimental design, the quadratic effect (a parabola, or “U-shaped” curve) would indicate a decrease in 5-HT$_2$ binding, returning to pre-treatment levels after a period of time, whereas the cubic effect would suggest that 5-HT$_2$ binding decreased significantly after treatment. A significant linear effect is suggestive of effects not related to treatment per se.
Results:

With one exception, seizure length decreased in all animals as the course of ECS progressed, even as the applied current was increased (Fig. 2.1, closed symbols, solid lines). That same animal showing no change in seizure length also required only a small increase in current over the 6 ECS sessions to continue to elicit long generalized seizures (Fig. 2.1, Monkey A, open symbols, dashed lines). Taking all of the animals in the study into account, applied current and seizure length were significantly negatively correlated (Pearson product-moment correlation, $r^2 = 0.18$, $p < 0.005$).

No significant differences were seen between the right and left hemispheres in any cortical region (one-way t-test), so data from each hemisphere were pooled. Figure 2.2 shows the changes in the $[^{18}F]$setoperone binding potential 24 hours, 1 week and 4-6 weeks after the last ECS treatment in all eight cortical regions examined. Two-way ANOVA with repeated measures revealed a significant effect of TIME ($F_{0.05(2), 3,126} = 43.25$, $p < 0.0001$). Post-hoc testing showed a significant decrease in $[^{18}F]$setoperone binding potential at 24 hours and 1 week post-treatment (Tukey test, $p < 0.0001$), but no significant differences between other time points. There was no significant effect of REGION. We investigated the timecourse of changes to 5-HT$_2$ binding further using polynomial contrasts, and found that the data for every brain region showed a significant quadratic effect (Table 2.1, Fig. 2.2). That is, in every region, 5-HT$_2$ binding was decreased from baseline at 24 hours and 1 week post-ECS, and gradually returned back to baseline by 4-6 weeks after the end of the ECS course, following a “U-shaped” curve.

![Figure 2.1: Relationship between seizure threshold and seizure length across the course of ECS. Six of the animals showed the typical increase in seizure threshold with concomitant decrease in seizure length (closed symbols, solid lines), while one animal (Monkey A) failed to show an increase in seizure threshold (open symbols, dashed lines). Data are presented as mean ± SEM.](image)

To examine the development of changes to 5-HT$_2$ binding during the course of ECS, five of the animals also received a PET scan 24 hours after the second or third treatment (depending
on scanner availability). Even after just two or three ECS treatments, three animals showed decreases in 5-HT₂ binding in most brain regions examined (Fig. 2.3A), while the fourth monkey showed little change or slightly increased 5-HT₂ binding early in the course of ECS. However, the fifth monkey (Monkey A) showed substantial increases in 5-HT₂ binding compared to baseline in every cortical region examined. Because only five animals were studied at this time point, we did not perform statistics on these data. For comparison, the results in all seven of the study animals at 24 hours after the last ECS are shown in parallel in Fig. 3B, and at this time point, the overwhelming effect was a decrease in 5-HT₂ binding in all brain regions examined, again with the exception of Monkey A.

Examination of the individual patterns for each animal over time revealed that one animal (Monkey A) did not show a reduction in [¹⁸F]setoperone binding in any brain region at 24 hours (Fig. 2.3B), 1 week or 4-6 weeks compared to pre-ECS values. Further examination of the data showed that this animal was also the one who did not have an elevated seizure threshold over the course of ECS (Fig. 2.1), and who showed increased binding in all brain regions examined 24 hours after the second or third ECS (Fig. 2.3A). It should be noted that exclusion of this animal in the ANOVA of time/treatment and regional effects does not change the overall outcome.
Figure 2.2: $[^{18}F]$Setoperone binding to 5-HT$_2$ receptors throughout the cortex in seven adult male rhesus monkey at baseline (pre-ECS), 24 hours, 1 week and 4-6 weeks after the last ECS session. Data are shown as the mean binding potential ± SEM for each region (where the binding potential is the region:cerebellum activity between 60 and 120 min post-injection minus one). 5-HT$_2$ binding was significantly decreased at 24 hours and 1 week post-ECS, but had returned to baseline at 4-6 weeks post-ECS (*Tukey test, p < 0.0001).
Table 2.1: Polynomial contrasts to determine the pattern of the changes in 5-HT$_2$ binding after a course of ECS. All brain regions show only a significant quadratic effect. (*p < 0.05)

<table>
<thead>
<tr>
<th>Region</th>
<th>F (linear)</th>
<th>F (quadratic)</th>
<th>F (cubic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Occipital cortex</td>
<td>0.01</td>
<td>18.80*</td>
<td>6.33</td>
</tr>
<tr>
<td>Parietal Cortex</td>
<td>0.62</td>
<td>17.29*</td>
<td>0.59</td>
</tr>
<tr>
<td>Central cortex</td>
<td>0.07</td>
<td>19.76*</td>
<td>0.44</td>
</tr>
<tr>
<td>Temporal Cortex</td>
<td>0.45</td>
<td>14.28*</td>
<td>0.39</td>
</tr>
<tr>
<td>Frontal Cortex</td>
<td>0.25</td>
<td>15.00*</td>
<td>0.06</td>
</tr>
<tr>
<td>Orbital Cortex</td>
<td>0.56</td>
<td>12.90*</td>
<td>0.16</td>
</tr>
<tr>
<td>Posterior Cingulate Cortex</td>
<td>1.24</td>
<td>8.60*</td>
<td>0.06</td>
</tr>
<tr>
<td>Anterior Cingulate Cortex</td>
<td>0.08</td>
<td>10.00*</td>
<td>0.01</td>
</tr>
</tbody>
</table>
Figure 2.3: Percent change in $[^{18}F]$_setoperone binding to 5-HT$_2$ receptors. A) The data from five monkeys 24 hours after two or three ECS treatments. B) For comparison, the data from all seven of the study animals 24 hours after the full course of ECS (six treatments). Each point represents one of eight cortical regions examined.
Discussion:

The main observation of this study is that monkeys treated with a clinical course of ECS show decreased binding to serotonin 5-HT$_2$ receptors throughout the cerebral cortex. Our data also show that changes in 5-HT neurotransmission may begin as early as the second or third ECS treatment, and that the decrease in 5-HT$_2$ binding persists for at least 1 week, but returns to baseline by 4-6 weeks after the last ECS treatment. The changes in 5-HT$_2$ binding in non-human primates after a course of ECS follow a quadratic (or “U” shaped) function, returning to baseline within days to weeks after the last treatment.

Although we primarily investigated the changes in 5-HT$_2$ binding at the end of the course of ECS, we did examine five animals 24 hours after the second or third treatment, and found that even a small number of ECS treatments can have substantial effects on 5-HT neurotransmission. Decreased [F]setoperone binding was already clearly evident in three of the five animals scanned at this time point in most brain regions (Fig. 2.3A), although to a lesser extent than after the full course of treatment (Fig. 2.3B). In humans, bilateral ECS gives an earlier onset of antidepressant effects than unilateral ECT (Sackeim et al., 1987a), and the fact that we employed bilateral ECS in this study may help explain the rapid changes detected in 5-HT$_2$ binding.

Taken together, our data provide physiological support for what has long been known by clinicians and patients alike, that the effects of ECT can emerge after only a few treatments, but that even with substantial improvements in mood after a full course of treatment, relapse is almost inevitable. To obtain an antidepressant effect, ECT is administered two to three times per week until there is a significant improvement in mood, typically requiring 6-12 treatments (American Psychiatric Association, 2001). Although the index course of ECT may be complete, the incidence of relapse is so high (approximately 50% at 6-12 months; Sackeim et al., 1993; Grunhaus et al., 1995; Bourgon and Kellner 2000), that continuation and/or maintenance therapy, either pharmacological or further ECT treatments, are standard practice. The timecourse and persistence of the effects of neither ECT nor ECS have been studied in detail. However, our data show that although the changes in 5-HT neurotransmission induced by a course of ECS begin to develop after only a few treatments, the effects are transient, and are not detectable 4-6 weeks after the cessation of treatment. The relative brevity of the neurochemical changes in our study (5-HT$_2$ binding returning to baseline within 4-6 weeks) should, however, be considered in light of two pieces of information: 1) our animals only received 6 ECS sessions, and 2) they were likely not depressed. Indeed, a clinical course of ECT is often comprised of more than 6 ECT sessions, which may increase the persistence of the effects on 5-HT$_2$ receptors, and the treatment is applied to an abnormally functioning 5-HT system, which may also prolong the effect. No matter what the exact time frame of the physiological response, the immediacy and transience of changes in 5-HT neurotransmission induced by ECS support the use of continuation and maintenance ECT treatments.

Our results are contrary to many previous studies in rodents of increased 5-HT$_2$ receptor binding and mRNA expression after chronic ECS (Kellar and Stockmeier 1986; Biegon and Israeli 1987; Burnet et al., 1995). There are several possible explanations for the disparate effects of electroconvulsive stimuli on 5-HT$_2$ receptors in the primate versus rodent brain, including differences in the method of administration (e.g. the typical lack of anaesthesia during the administration of ECS to rodents, or differences in schedule, current, or electrode placement) or simply species differences in metabolism, neuroanatomy and neurochemistry. However, in rodents and humans, chronic treatment with many classes of antidepressant drugs, including tricyclics, monoamine oxidase inhibitors, some SSRIs (with the notable exception of fluoxetine), and atypical antidepressants, downregulation of 5-HT$_2$ receptors appears to be the predominant effect (Bergstrom and Kellar 1979; Peroutka and Snyder 1990; Todd et al., 1995; Attar-Levy et al., 1999; Yatham et al., 1999; Mischoulon et al., 2002, Strome et al., 2005). Synthesizing the
literature, it appears that it is the enhanced presence of 5-HT$_2$ receptors in rodents after ECS that is the anomaly, and that in fact, widespread downregulation of 5-HT$_2$ receptors may be a common effect of most antidepressant treatments.

The mechanism of action of ECT and other antidepressant treatments is not entirely understood, but enhancement of monoamine neurotransmission is likely an integral part of the antidepressant effect. In vivo microdialysis studies in rats suggest that hippocampal interstitial 5-HT concentration increases dramatically after a single ECS (Zis et al., 1992), as does the frontal cortex concentration of the major metabolite of 5-HT, 5-HIAA, after both acute and chronic ECS (Yoshida et al., 1998). In vitro studies in rodents following chronic antidepressant drug treatments generally show reduced 5-HT$_2$ receptor density, with no alterations in the affinity of the receptors (Cross and Horton 1988; Newman et al., 1990), likely a compensatory effect for the sustained elevation of synaptic 5-HT resulting from chronic blockade of the reuptake transporter. A similar mechanism may also underlie our current results. If, in our study animals, ECS led to an enhancement of synaptic 5-HT levels, then downregulation of 5-HT$_2$ receptors may have occurred as a compensatory response to chronically elevated 5-HT. As the levels of 5-HT slowly returned to baseline after cessation of ECS, so too would 5-HT$_2$ receptor binding. A similar mechanism has been demonstrated in another monoamine system, dopamine, where chronic changes in endogenous ligand concentration cause changes in dopamine receptor density (Rinne et al., 1995; Doudet et al., 2002).

Alternatively, our finding of decreased 5-HT$_2$ receptor binding 24 hours and 1 week after a course of ECS may be explained by competition between the tracer, setoperone, and the endogenous ligand, 5-HT. Decreased tracer binding as a consequence of competition from increased synaptic concentrations of the endogenous ligand has been described for dopaminergic tracers of the benzamide family such as raclopride or iodobenzamide (Laruelle 2000; Doudet and Holden 2003). It is unknown if setoperone is sensitive to competition by endogenous 5-HT, and as such, this hypothesis cannot be excluded. However, a recent study (Hirani et al., 2003) suggests that other selective 5-HT$_2$ receptor ligands such as MDL 100,907 are not displaced by increased synaptic concentrations of 5-HT. Thus, in the absence of competition studies with setoperone, especially in vivo microdialysis and Scatchard-type studies, it is premature to draw a conclusion on the origin and nature of the observed decrease in 5-HT$_2$ binding.

As is frequently observed during treatment of human patients with ECT (Sackeim et al., 1987b; Mukherjee 1989; Coffey et al., 1995), we found that in general, seizure threshold increased as the course of ECS progressed, while at the same time, seizure length decreased. This phenomenon is well documented, and is accredited to the anticonvulsant effects of repeated seizure induction. Seizures are believed to be due, at least in part, to excessive, uncontrolled firing in excitatory glutamatergic cortical neurons. Although the actual mechanism of the anticonvulsant effect is unknown, proposed contributors include increased release and/or activity of inhibitory factors such as opiates (Nakajima et al., 1989), thyrotropin-releasing hormone (Post et al., 2000), and γ-aminobutyric acid (GABA; Sanacora et al., 2003). Whatever the cause of this anticonvulsant effect, it may represent an adaptive, protective mechanism, to limit cell death resulting from excessive glutamate release and subsequent excitotoxicity. Moreover, our observation of this effect in non-human primates during a course of ECS suggests that non-human primates provide an appropriate model to study the neurochemical effects of ECT.

Although the anticonvulsant effect of ECT is widely recognized, seizure threshold and duration may be distinct characteristics. Several factors can influence the seizure threshold and duration, but these are not always similarly affected. For example, the administration of pharmacological agents can either increase or decrease seizure length (e.g. with caffeine and benzodiazepines respectively) without necessarily altering seizure threshold (reviewed in Datto et al., 2002). In comparison, placement of the electrodes in the bilateral compared to unilateral
position increases the seizure threshold, but not the length (Sackeim et al., 1987a). Indeed, as far as clinical efficacy is concerned, seizure threshold may be the more predictive variable. The consistent observation of increased seizure threshold with subsequent treatments led to the anticonvulsant hypothesis of the mechanism of action of ECT (Sackeim et al., 1983; 1987b). While by no means unequivocal, this hypothesis suggests that the anticonvulsant properties of ECT are directly related to its clinical efficacy, and is supported by several studies showing that a rise in seizure threshold is a necessary but insufficient requirement for clinical response to ECT (Sackeim et al., 1983, 1987b; Mukherjee 1989). In this view, it is notable that the only animal in our cohort whose seizure threshold did not increase throughout the course of ECS was also the only animal in which [18F]setoperone binding was unaffected. While early in the course of ECS this animal (Monkey A) showed increased binding in all brain regions examined (Fig. 2.3A), [18F]setoperone binding was generally within the test-retest reproducibility (about 10%) of the technique at all other time points compared to baseline (e.g. Fig. 2.3B). To the best of our knowledge, there was nothing "clinically" different about Monkey A: he was a normal control, dominant animal, and although we do not have information about his specific rearing conditions, he had been living in the same conditions and environment as the other animals in this study for over 13 years. He may simply represent the non-human primate counterpart of a human patient who does not respond to ECT. Presuming changes in the structure and/or function of neural systems underlie the positive effects of ECT, changes in seizure expression may be the overt representation of those neurobiological correlates.

The last point to consider in the interpretation of our data is the use of anaesthesia for both the ECS treatment and the PET imaging. The ECS treatments were performed in ketamine-anaesthetized animals. Although short-acting barbiturates are the anaesthetic of choice for human ECT, the necessity of intravenous administration makes them difficult to use in older non-human primates, where venous access can be limited. We opted to use ketamine based on two factors. Ketamine is the preferred anaesthetic for immobilizing non-human primates prior to handling, and is easily and safely administered intramuscularly. Secondly, ketamine is the favoured alternative to methohexital or propofol anaesthesia in human patients with allergies to barbiturates, or in those patients who require modified ECT to increase seizure duration (Rasmussen et al., 1996). Compared to methohexital, ketamine anaesthesia for ECT results in increased seizure duration, reduced reorientation time, and increased EEG ictal amplitude, indicative of more widespread seizure activity (Krystal et al., 2003). In rodents, one of the unique effects of ECS compared to other antidepressant treatments is its robust induction of hippocampal mossy fibre sprouting (Vaidya et al., 1999), and although one report comparing the effects of ECS in rodents under ketamine or no anaesthesia suggests that the degree of hippocampal mossy fibre sprouting is reduced under ketamine (Chen et al., 2001), the induction of sprouting is still significantly greater than in sham treated animals. Similarly, in non-human primates treated with ECS under ketamine anaesthesia, both mossy fibre sprouting and neurogenesis in the dentate gyrus are increased relative to sham-treated controls (Scalia et al., 2003). Thus the relevance of our data to the mechanism of action of ECT is unlikely to be invalidated by the use of ketamine anaesthesia. Another potential confound is the use of isoflurane gas as the anaesthetic for the PET studies. Most human in vivo and rat in vitro receptor binding studies are performed without anaesthesia. However, all the animals were scanned under the same anaesthesia conditions at all four time points. Thus, the effects of anaesthesia may be negligible in the interpretation of the longitudinal data.

In conclusion, this study is the first to show widespread downregulation of 5-HT2 binding throughout the non-human primate brain as a result of chronic ECS treatment. This finding is in agreement with previous reports of the effects of antidepressant treatments in depressed patients, and in animals, and suggests that 5-HT2 receptor downregulation may be a common effect of
antidepressant treatments. As hypoactivity among serotonergic neurons is implicated in the etiology of human depression, changes in receptor binding after ECT may be reflective of a compensatory change in response to enhanced 5-HT tone. Although we did not use an animal model of depression to investigate the development and persistence of the effects of ECS on 5-HT₂ receptors, the similarity of our results with those of other studies examining the effects of antidepressant treatments on 5-HT₂ receptors in depressed individuals suggests that ECT in human patients also decreases 5-HT₂ binding. ECT is one of the most effective antidepressant treatments currently available, and our results provide even further support for its continued use and study.
References:


Yates M, Leake A, Candy JM, Fairbairn AF, McKeith IG, Ferrier IN (1990) 5HT₂ receptor changes in major depression. Biol Psychiatry 27: 489-496.


Chapter 3


Preamble:

In the previous chapter, we studied the effects of repeated ECS treatment on 5-HT$_2$ receptors in the cerebral cortex of non-human primates using PET. While the effects were robust and clear, this study also identified several issues regarding the feasibility of performing our investigations into the mechanism of action of ECT in PD using non-human primates and PET. To further investigate the effects of repeated ECS on the DA system, we decided to move away from non-human primates, and instead adopted a rat model. Even though we changed our animal model, we wanted to continue to use the same molecular probes that we had used in vivo in our PET studies in non-human primates. While it is possible to perform PET imaging in small animals using a microPET scanner (see Chapter 4, for example), this technique has its own inherent limitations, and at the time that we began these studies we did not have access to a microPET scanner. This chapter describes, in detail, a technique that we developed to perform quantitative autoradiographic receptor binding assays in vitro using PET tracers. As in the previous chapter, we measured binding to 5-HT$_2$ receptors as our neurochemical endpoint, but instead of looking at repeated ECS treatment, here we used repeated treatment with the antidepressant desipramine because this pharmacological challenge decreases 5-HT$_2$ receptor binding in both rodents and humans (Bergstrom and Kellar, 1979; Goodnough and Baker, 1994; Yatham et al., 1999).

Introduction:

Receptor autoradiography is a widely used technique to determine the characteristics and distribution of neurotransmitter receptors. Traditionally, in vitro receptor binding assays are performed on tissue slices with tritiated ligands and x-ray film, or with membrane homogenates and liquid scintillation counting. Phosphor imaging autoradiography has recently emerged as a sensitive and widely available alternative with several advantages over x-ray film autoradiography.

The most important advantage of phosphor imaging over film autoradiography is temporal. The high sensitivity of phosphor screens allows exposure times to be reduced to one-tenth of x-ray film exposure times (Johnston et al., 1990), allowing data to be collected and analyzed in hours or days instead of weeks. Another advantage of phosphor screens over film is economy. Multisensitive phosphor screens can be used repeatedly, by erasing any latent image remaining after scanning by direct exposure to white light. With the added care and precautions reported here for the use of tritium-sensitive phosphor screens, we have been able to use these screens at least seven times to obtain reliable quantitative data. Phosphor imaging also provides a practical alternative, as tritium-sensitive x-ray film is becoming increasingly difficult to obtain.

Historically, autoradiography with positron emission tomography (PET) ligands has been very difficult to perform due to the short half-lives of positron-emitting isotopes and the low sensitivity of x-ray film. Phosphor screens have higher sensitivity, and good, quantifiable images can be obtained from PET tracers within the naturally short exposure times. In vitro

---

autoradiography with PET ligands is an important adjunct technique for corroborating in vivo PET data, and the ability to use the exact same ligand for in vitro studies as is used in vivo permits closer comparisons. Recently, PET groups have begun to add in vitro phosphor imaging with $[^{11}C]$- or $[^{18}F]$-labelled compounds as an adjunct to in vivo imaging, and the technique has an important role in tracer discovery (Bergstrom et al., 2004). Qualitative anatomical distribution studies are common, often performed ex vivo, where the animal is injected with the radiotracer, sacrificed after tracer uptake, and tissue slices are apposed to the storage phosphor screens (e.g. Besret et al., 1998; Passchier et al., 2000, Doze et al., 2002). Phosphor imaging with PET tracers has also been used with live tissue preparations, where the tissue is removed from the animal but maintained in biological buffer solutions with oxygenation during incubation with the PET compound (Matsumura et al., 1995, Sasaki et al., 2002). Very few studies have used in vitro phosphor imaging with PET tracers for quantitative autoradiography (Gatley et al., 1998; Nikolaus et al., 2001; 2003).

We have used a routine pharmacological challenge to demonstrate and validate the use of PET tracers and phosphor imaging for quantitative in vitro receptor autoradiography. Chronic antidepressant treatment consistently leads to decreased binding to cortical serotonin-(5-HT)$_2$ receptors. This effect has most often been demonstrated with the tricyclic antidepressant desipramine hydrochloride (DMI), which decreases 5-HT$_2$ receptor binding in both humans and animals (Bergström and Kellar, 1979; Goodnough and Baker, 1994; Yatham et al., 1999). The effect is robust and has been detected with various 5-HT$_2$ ligands, both in vivo by PET, and in vitro using membrane preparations. To the best of our knowledge, however, decreased 5-HT$_2$ binding as a result of chronic DMI treatment has not been investigated by slice autoradiography, which has much higher spatial resolution than homogenate binding assays. Here, using the effects of chronic DMI treatment on 5-HT$_2$ binding as the validation end-point, we describe aspects of the method critical to the acquisition of accurate quantitative autoradiographic phosphor imaging data in vitro, using both the traditional, tritiated 5-HT$_2$ ligand, $[^3H]$ketanserin, and the positron-emitting ligand $[^{18}F]$setoperone.

**Materials and Methods:**

**Subjects**

Adult male Sprague-Dawley rats (n = 5 per group), 300 g at the start of the study, were the subjects of this experiment. Rats were housed on a 12:12 light-dark cycle, with lights on at 6 a.m. They were housed in dyads with food and water available ad libitum. The rats were given 15 mg/kg i.p. DMI (Sigma) or a similar volume of vehicle (sterile water) daily for 17 d. On the 18th day, the animals were decapitated, and their brains were quickly removed and frozen in isopentane cooled with dry ice, and stored at -80°C until sectioning. Twenty micron coronal sections were cut throughout the brain on a cryostat set to -18°C and thaw mounted onto Superfrost Plus slides (Fisher Scientific). The slides were re-stored at -80°C until the binding studies were performed.

**5-HT$_2$ Binding: $[^{18}F]$Setoperone**

The details of the radiosynthesis have been reported elsewhere (Adam et al., 1997). The data were acquired on two different days using the same procedure. The slides were warmed up to room temperature, and pre-washed for 15 min in 50 mM Tris-HCl buffer (50 mM Trizma HCl, 120 mM NaCl, 5 mM KCl, 1 mM MgCl$_2$, 2 mM CaCl$_2$, pH 7.4, 25°C). They were incubated with 9 nM $[^{18}F]$setoperone (day 1 specific activity 265 Ci/mmoll, day 2 specific activity 1214 Ci/mmoll at the time of incubation) in the same buffer at 25°C for 1 h. Non-specific binding was performed by incubating adjacent slices with an additional 2 μM ketanserin
At the end of the incubation, the slides were washed for 2 x 10 min in fresh buffer at 4°C, dipped in deionized, distilled water (4°C), and allowed to air dry in a fumehood for 20 min.

While the slides were incubating and drying, a set of $^\text{[18F]}$-standards was created following the technique of Gatley et al. (1998) by pipetting 5 μL drops of a set of 8 serial dilutions onto a 25 x 75 mm strip of TLC plate (PE SIL G, Whatman). The highest concentration in the serial dilutions was the same as the incubation concentration (9 nM), but our studies with other radioligands indicate that this is not necessarily appropriate for every radioligand, and must be determined for each ligand, and under each set of binding conditions.

The Multisensitive phosphor screens (Perkin-Elmer) were gently cleaned with Intensifying Screen Cleaner (Kodak) and placed against a standard radiology light box immediately before use to erase any background radiation that may have been detected while in storage. The slides and TLC strips were apposed to the phosphor screens in standard film cassettes with lead shielding around and between the cassettes. Our initial experiments indicated that it is necessary to expose each screen with its own internal standard (TLC plate) due to minor inherent differences in the sensitivities of the screens and variations in background noise. After 20 h of exposure at room temperature, the screens were removed from the cassettes under dim lighting and immediately scanned at 600 dpi resolution using a Cyclone storage phosphor system (Perkin-Elmer), and the data analyzed using the system’s Optiquant v4.00 software.

5-HT$_2$ Binding: $^\text{[3H]}$Ketanserin

The $^\text{[3H]}$ketanserin binding assay was performed according to Lopez-Gimenez et al. (1997). Briefly, slides were warmed up to room temperature, and pre-washed for 15 min in 170 mM Tris-HCl buffer (pH 7.7). The slides were incubated in 2 nM $^\text{[3H]}$ketanserin (Perkin-Elmer; specific activity 88 Ci/mmol, 97% pure by HPLC) in the same buffer at 4°C for 120 min. Non-specific binding was performed by incubating consecutive slices with an additional 1 μM mianserin. At the end of the incubation, the slides were washed for 2 x 10 min in fresh buffer at 4°C. After post-fixation in paraformaldehyde (Sigma) vapour for 2 d (Liberatore et al., 1999), the slides were apposed to tritium-sensitive phosphor screens (BAS-TR2025 Fuji Medical Systems Inc., Stamford, CT) in standard film cassettes with $^\text{[3H]}$microscales (Amersham, UK) for 3 d at room temperature. As with the Multisensitive screens, the tritium-sensitive screens were placed against a light box to erase any latent image before use. On the third day, under dim lighting, the screens were removed from the cassettes and scanned immediately in the phosphor imager at 600 dpi resolution.

$^\text{[18F]}$Setoperone Standard Curves

For each point on the standard curve, the image was displayed relative to that point before encompassing the region within a circular region of interest (ROI) to obtain an optical density measurement in digital light units (DLU). This step is necessary when there is a wide dynamic range in the image because when the image is displayed in the default mode adopted by the software, which excludes pixels at the extremes of the distribution, very hot spots can spillover onto neighbouring pixels, whereas the coldest spots in the curve can be lost visually (see results section below and Fig. 3.1). This can lead to overestimation of the relative optical density (ROD) in the hottest spots, and underestimation in the coldest spots, and hence inaccuracies in the standard curve itself. After the optical density readings were taken for each standard spot, background was subtracted by measuring the DLU in an equivalent, but unexposed area near the standards. The standard curves were created using GraphPad Prism v3.00 software and linear regression analysis, because unlike film, phosphor screens have a wide linear dynamic range (Johnston et al., 1991; Upham and Englert, 1998). Because the spots
consisted of a known amount of activity, and were measured on a presumably two-dimensional surface (the TLC plate), the standard curves related ROD (in DLU) to activity (in nCi).

\[18\text{F}]\text{Setoperone Binding Analysis}

Serotonin 5-HT\textsubscript{2} binding was determined in the prefrontal, frontal, occipital and anterior, middle and posterior parietal cortices. For each region, one small elliptical ROI (area = 0.5 mm\textsuperscript{2}) was placed per hemisphere on two total and one non-specific binding section for each animal. During the placement of ROIs, the image was displayed relative to the area under investigation, both to increase the accuracy of the placement by removing any spillover effect, and to ensure that the analyst was blind to the treatment condition. A background ROI of the same area was also placed adjacent to each slide. The ROD of each ROI was measured in DLU, and the appropriate background was subtracted from each ROI. Mean non-specific binding was subtracted from mean total binding to give a mean specific binding measure for each subject. This value was then placed against the standard curve for the screen that it was derived from, and the corresponding activity was determined. Data were then converted to nCi/cc of tissue, assuming that the volume of the ROI was a product of the area (0.5 mm\textsuperscript{2}) and the section thickness (20 \textmu m). Variations in the specific activity of PET tracers arise naturally during the synthesis of the tracer on different days. In in vitro receptor binding assays it is important that the concentration of the ligand remains constant across assays. The amount of activity added to the incubation is therefore different on different days, depending on the specific activity. To correct for this, one must divide the specific binding data for each subject (in nCi/cc) by the specific activity at the start of incubation (in Ci/mm) to get a measure of receptor binding in pmol/cc.

\textbf{Resolution of \([18\text{F}]\text{Phosphor Imaging}}

To examine the spatial resolution of \([18\text{F}]\text{phosphor imaging, we produced five series of point sources of different diameters, printed with \([18\text{F}]\text{-ink injected into the ink cartridge of a standard Hewlett-Packard Inkjet printer on regular printer paper (Fig. 3.2). The characteristics of the point sources are indicated in table 3.1. After exposing the point sources to a Multisensitive phosphor screen for 3 h, the screens were scanned with the phosphor imager as described above. The image file was exported into the software program Matlab 6.0 (The MathWorks, Natick, MA) for further analysis. Using the smallest diameter spots, we took the average intensity values in a column a few pixels wide in the middle of each spot, in both the horizontal and vertical directions, and fitted a Gaussian distribution to the resultant point-spread function (PSF) to determine the resolution of the system in terms of full-width at half-maximum (FWHM). The resolution is defined by the formula:}

\textbf{Equation 3.1: Actual FWHM}_{PSF} = \sqrt{(\text{Measured FWHM}_{PSF})^2 - (\text{Source size})^2}

where source size is the diameter of the point source. In a true point source, the second term is infinitesimally small, and actual FWHM = measured FWHM. However, in our case, the point source has a non-negligible diameter (0.64 \textmu m), which must be taken into account.
Figure 3.1: The same $^{18}$F standard displayed three different ways, illustrating the spillover effect. The standards are displayed A) relative to the entire image (default), B) relative to the hottest spot (far left - arrow), and C) relative to the coldest spot (far right - arrow). Note how the area of the spot changes depending on how the image is displayed (particularly the hottest spots). However, each spot is derived from the same volume of activity (5 µL), and when the image is displayed relative to the spot in question, the areas are standardized - the hottest spot in image B (arrow) has a similar area to the coldest spot in image C (arrow).
Table 3.1: Characteristics of the point source series used to create Figure 3.2.

<table>
<thead>
<tr>
<th>Diameter (mm)</th>
<th>Distance Center-to-Center (mm)</th>
<th>Activity of each spot (nCi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.64</td>
<td>2.56</td>
<td>1.54</td>
</tr>
<tr>
<td>0.95</td>
<td>3.80</td>
<td>3.86</td>
</tr>
<tr>
<td>1.65</td>
<td>6.60</td>
<td>6.95</td>
</tr>
<tr>
<td>2.41</td>
<td>9.64</td>
<td>13.90</td>
</tr>
<tr>
<td>3.30</td>
<td>13.20</td>
<td>26.25</td>
</tr>
</tbody>
</table>

[^3H]Ketanserin Binding Analysis

For the [^3H]ketanserin experiment, the data were analyzed in a similar manner to the [^18F]setoperone experiment. However, since the standard curves were derived from commercial[^3H]microscales, which are calibrated for 20 μm tissue sections, the standard curves relate nCi/mg tissue to DLU/mm$^2$, and both the tissue volume and tracer specific activity corrections applied to the[^18F]setoperone data are unnecessary for the[^3H]ketanserin data. For comparison with the[^18F]setoperone data, the[^3H]ketanserin data were converted to pmol/cc knowing the specific activity (88 Ci/mmol), and with the assumption that brain tissue has a density of 1.0 g/cc and the conversion factor:

Equation 3.2: $1 \text{nCi/mg} = 1 \times 10^{-9} \text{Ci} / 1 \times 10^{-3} \text{cc} \div 88 \text{Ci/mmol} \times 1 \times 10^9 = 11.36 \text{pmol/cc}$

Statistical Analysis

All statistics were performed with either GraphPad Prism v.3.0 or STATISTICA '98 (StatSoft, Tulsa, OK) software. The data collected using[^18F]setoperone at low and high specific activities on two different days, and the data from the two different tracers were compared using a paired t-test. For examination of treatment and regional effects, data were compared with two-way analysis of variance with treatment (DMI vs. vehicle) and cortical region as the factors.
Figure 3.2: Point sources printed on paper with $^{18}$F ink. A) Actual phosphor images of the point sources used to examine the spatial resolution of the Multisensitive phosphor screens. The point sources are 3.30, 2.41, 1.65, 0.95 and 0.64 mm in diameter, and the distance between each spot is four times the diameter center-to-center. B) A representative point-spread function, determined from the smallest diameter point sources in A). The X-axis has been normalized to mean ± SD = 0 ± 1. After fitting to a Gaussian distribution, and knowing the pixel size of the system, we estimate the spatial resolution of the system to be about 470 μm FWHM.
Results:

I. Technical Issues of \(^{18}\)F Phosphor Imaging

Fit and Reproducibility of \(^{18}\)F Standard Curves: The Spillover Effect

Displaying the image relative to the spot in question ("spillover correction") when determining the optical density of a particular point on the standard curve is critical to the production of accurate standard curves with excellent goodness of fit and high reproducibility. Because the standards are created on a two-dimensional surface, where the known value is the amount of activity in each spot, and because the shapes of the standard spots and distribution of activity within the spot can vary, the entire area must be taken into account to create the standard curve. This means that inaccurate delineation of the standard spot can greatly affect the fit of the standard curve, and consequently the quantification of the data.

Figure 3.3A shows an example of what the curve looks like after spillover correction (squares) compared to if the optical density is measured when the image is displayed in the default mode (triangles). The fit of the line is better after spillover correction (Linear regression: squares $r^2 = 0.99$, $S_{yx} = 0.99$, triangles $r^2 = 0.99$, $S_{yx} = 1.24$). By displaying the image relative to the spot in question, we remove spillover in the hottest spots, and are better able to delineate the coldest spots, thereby defining the standard spots more accurately. Equally importantly, Figs. 3.3B and 3.3C illustrate how spillover correction reduces the variability across standards made on a single day (Fig. 3.3B mean $r^2$, $S_{yx} = 0.99$, 5.62, Fig. 3.3C mean $r^2$, $S_{yx} = 0.99$, 2.78).

Not only does spillover correction of the standard curves decrease the variability across standards made on a single day, but it also decreases variability across days. 3.4A shows the mean values of the standard curves produced on two different days without spillover correction, and Fig. 3.4B shows the same curves after spillover correction.

Because the software default displays the image with pixel values at the extremes of the distribution removed, and because pixels of high activity "spillover" onto neighbouring pixels, the ROD in an ROI must also be determined when the image is displayed relative to the region in question (so that the minimum and maximum pixel values are also those of the region in question). Correction for spillover also helps in determining the appropriate size of the ROIs.

Resolution of \(^{18}\)F Phosphor Imaging

Figure 3.2A shows a representative image from the point source experiments. The resolution of the software and printer were the limiting factors in this experiment, and we could not accurately produce smaller diameter spots. Points in every diameter series were easily visually resolvable. Each point source produced a Gaussian activity distribution (Fig. 3.2B). Based on the mean distribution of the 15 point sources from the smallest series of points (diameter = 0.64 mm), we estimate the resolution to be about 470 μm full-width at half-maximum (FWHM; table 3.2).
Figure 3.3: Fit of the curves derived from the $[^{18}F]$ standards. A) After spillover correction of the data in Fig. 3.1, by displaying the image relative to each point in the curve before taking optical density readings, the data are a better fit to the standard curve (squares) than if optical density is measured without partial volume correction (triangles). B) Standard curves created on the same day, but quantified without spillover correction. C) The same set of curves as in B), but with spillover correction. By compensating for spillover, a set of standards created on the same day, but apposed to different screens shows both a better fit to the points in the curves, and less variability in slope.
Figure 3.4: Spillover correction decreases the variability of the slopes of standard curves from different days. A) Mean standard curves from two different days measured without spillover correction. B) Mean standard curves from two different days measured with spillover correction.

Table 3.2: Determination of the spatial resolution of the Multisensitive phosphor screens. Point-spread functions were determined for each of the spots in the smallest diameter series, and fit to a Gaussian distribution. From the FWHM of the PSF, the known diameter of the point source, and the known pixel size of 42.3 μm, we estimate the resolution of the system to be about 470 μm.

<table>
<thead>
<tr>
<th>Direction of measurement</th>
<th>Mean ± SD PSF FWHM (μm)</th>
<th>Diameter of the point source (μm)</th>
<th>Resolution (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horizontal</td>
<td>789.14 ± 23.89</td>
<td>640</td>
<td>461.67</td>
</tr>
<tr>
<td>Vertical</td>
<td>798.59 ± 25.49</td>
<td>640</td>
<td>477.65</td>
</tr>
<tr>
<td>Both</td>
<td>793.86 ± 24.33</td>
<td>640</td>
<td>469.70</td>
</tr>
</tbody>
</table>
II. Technical issues of [³H]phosphor imaging

Reusability of Tritium-Sensitive Phosphor Screens

Although tritium-sensitive phosphor screens lose their sensitivity over time (due to moisture accumulation), the loss of sensitivity can be corrected for to a certain extent. The accumulated moisture affects the screen homogeneously, in the form of increased background noise. We examined the loss of sensitivity by exposing one tritium-sensitive phosphor screen to the same set of slides in two consecutive exposures. Figure 3.5A shows the data from several background regions after a 3 d exposure upon first removing the screen from the shrink-wrap packaging that it is shipped in, and after a subsequent 3 d exposure (scanned 6 d after opening). There is a significant increase in the optical density (DLU/mm²) of the background regions ($t_{0.05(2),12} = 4.75$, $p < 0.0005$). However, after correcting the data from the ROIs placed on brain tissue slices collected on those two consecutive exposures by subtracting the appropriate background regions, there are no significant differences between the data collected on the two days (Fig. 3.5B; $t_{0.05(2),12} = 0.09$, $p > 0.93$).

To analyze the reusability of the tritium-sensitive phosphor screens over even longer time periods, we apposed the plastic [³H]microscales to the screens at several time-points after the screens were first opened. After decay correction, linear regression analysis of the mean DLU/mm² values from eight microscales, indicates that the response of the screens remains linear for at least three months after the first use, but that the slopes of the lines are significantly different (Fig. 3.6A; linear regression $r^2 = 1.0$ for all; analysis of covariance (ANCOVA) $F_{0.05(2), 3,24} = 5.47$, $p < 0.005$). However, when background is subtracted from the data, there is no significant difference between the slopes of the standard curves (Fig. 3.6B; ANCOVA $F_{0.05(2), 3,24} = 2.42$, $p > 0.09$).

![A)](image)

![B)](image)

Figure 3.5: Background subtraction corrects for repeated use of tritium sensitive phosphor screens. A) Data from several background regions obtained from the same tritium-sensitive phosphor screen 3 and 6 days after opening. There is a significant increase in background noise with subsequent exposures ($p < 0.0005$). DLU = digital light units. B) After subtracting the appropriate background regions from the ROIs placed on the tissue slices, there is no significant difference between the two exposures.
Figure 3.6: Background subtraction compensates for accumulation of moisture by tritium-sensitive phosphor screens for up to three months after first opening. A) Data from a set of eight $[^{3}\text{H}]$microscales apposed to the phosphor screens at several time points without background subtraction. Even after three months since the first exposure, the response remains linear. However, the slopes of the lines are significantly different. B) The same data with background subtracted from each point on the standard curve. There is now no significant difference between the slopes of any of the lines.

III. Effects of DMI Treatment on 5-HT$_2$ Binding

Treatment and Regional Effects

Chronic treatment with DMI decreased 5-HT$_2$ binding in all cortical regions examined, as measured by both $[^{3}\text{H}]$ketanserin and $[^{18}\text{F}]$setoperone. The tissue for all three binding studies ($[^{3}\text{H}]$ketanserin and $[^{18}\text{F}]$setoperone low and high specific activity) came from the same ten animals ($n = 5$ per group), using consecutive slides for the examination of regional effects. The placement of ROIs was standardized for a particular area of cortex, and was consistent across all three binding studies. Table 3.3 shows the corrected binding data for several cortical regions collected on two different days, using low and high specific activity $[^{18}\text{F}]$setoperone. The data for each region were compared with a paired t-test, and no significant differences were found. Therefore, in regions where binding was performed on both days, the mean binding value for each animal was used for further analyses (frontal and anterior and posterior parietal cortices) (table 3.4).

Two-way analysis of variance (ANOVA) with repeated measures indicates $[^{18}\text{F}]$setoperone binding was significantly decreased by chronic DMI treatment in all cortical regions examined ($F_{0.05(2), 1.7} = 42.16$, $p < 0.0003$), and that there were significant regional differences ($F_{0.05(2), 5.35} = 56.95$, $p < 0.0001$). Post-hoc testing (Scheffe test) indicates that binding in the prefrontal and occipital cortices was different than all other regions. There was also a significant treatment/region interaction ($F_{0.05(2), 5.35} = 3.85$, $p < 0.007$). The right panel of table 3.4 shows the $[^{3}\text{H}]$ketanserin data. Again, chronic DMI treatment significantly decreased 5-HT$_2$ binding in all cortical regions examined ($F_{0.05(2), 1.7} = 13.65$, $p < 0.008$), and again, there was a significant regional effect ($F_{0.05(2), 5.35} = 21.41$, $p < 0.0001$), with post-hoc testing indicating significantly lower binding in the occipital cortex than all other regions, and higher binding in the prefrontal cortex than the posterior parietal and occipital cortices. There was also a significant treatment/region interaction ($F_{0.05(2), 5.35} = 3.04$, $p < 0.02$).
Table 3.3: Decreased 5-HT$_2$ binding after chronic treatment with DMI detected using $[^{18}\text{F}]$setoperone. 5-HT$_2$ binding was significantly decreased throughout the cerebral cortex. After correcting for differences in specific activity, the data from two different days are not significantly different.

<table>
<thead>
<tr>
<th>Region</th>
<th>DMI</th>
<th>VEH</th>
<th>% change</th>
<th>DMI</th>
<th>VEH</th>
<th>% change</th>
<th>Paired t-test p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prefrontal (+3.7)$^a$</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>71.70 ± 10.18</td>
<td>115.2 ± 15.22</td>
<td>-37.76</td>
<td>-</td>
</tr>
<tr>
<td>Frontal (+1.7)</td>
<td>56.3 ± 6.71</td>
<td>82.69 ± 19.01</td>
<td>-31.91</td>
<td>50.94 ± 5.49</td>
<td>79.20 ± 8.18</td>
<td>-35.68</td>
<td>0.27</td>
</tr>
<tr>
<td>Anterior parietal (+0.7)</td>
<td>59.43 ± 5.80</td>
<td>87.41 ± 13.02</td>
<td>-32.01</td>
<td>58.82 ± 6.63</td>
<td>93.95 ± 8.64</td>
<td>-37.39</td>
<td>0.51</td>
</tr>
<tr>
<td>Parietal (-1.3)</td>
<td>55.41 ± 7.98</td>
<td>86.05 ± 15.62</td>
<td>-35.61</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Posterior parietal (-2.3)</td>
<td>49.74 ± 12.83</td>
<td>69.42 ± 14.57</td>
<td>-28.35</td>
<td>48.81 ± 6.01</td>
<td>72.08 ± 13.90</td>
<td>-32.28</td>
<td>0.86</td>
</tr>
<tr>
<td>Occipital (-5.8)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>25.75 ± 4.78</td>
<td>34.05 ± 7.81</td>
<td>-24.38</td>
<td>-</td>
</tr>
</tbody>
</table>

Data are mean ± SD. $^a$numbers in parentheses are the coordinates in mm from Bregma (Paxinos and Watson, 1997). DMI = desipramine hydrochloride 15 mg/kg i.p for 17d, VEH = vehicle (sterile water).

Table 3.4: Correlation between $[^{18}\text{F}]$setoperone and $[^{3}\text{H}]$ketanserin binding. After pooling the data from the $[^{18}\text{F}]$setoperone studies, the data obtained with the PET and tritiated tracers are equivalent.

<table>
<thead>
<tr>
<th>Region</th>
<th>$[^{18}\text{F}]$setoperone binding pooled (pmol/cc)$^b$</th>
<th>$[^{18}\text{F}]$setoperone binding pooled (pmol/cc)$^b$</th>
<th>$[^{3}\text{H}]$ketanserin binding (pmol/cc)$^c$</th>
<th>$[^{3}\text{H}]$ketanserin binding (pmol/cc)$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prefrontal (+3.7)$^a$</td>
<td>71.70 ± 10.18</td>
<td>115.2 ± 15.22</td>
<td>59.55 ± 16.27</td>
<td>98.49 ± 17.19</td>
</tr>
<tr>
<td>Frontal (+1.7)</td>
<td>53.66 ± 5.41</td>
<td>80.95 ± 11.90</td>
<td>46.20 ± 8.45</td>
<td>85.67 ± 30.53</td>
</tr>
<tr>
<td>Anterior parietal (+0.7)</td>
<td>59.13 ± 5.36</td>
<td>90.68 ± 5.84</td>
<td>60.23 ± 15.62</td>
<td>84.92 ± 20.21</td>
</tr>
<tr>
<td>Parietal (-1.3)</td>
<td>55.41 ± 7.98</td>
<td>86.05 ± 15.62</td>
<td>58.55 ± 17.98</td>
<td>82.80 ± 26.25</td>
</tr>
<tr>
<td>Posterior parietal (-2.3)</td>
<td>49.28 ± 6.93</td>
<td>70.75 ± 11.05</td>
<td>46.89 ± 6.37</td>
<td>65.38 ± 22.54</td>
</tr>
<tr>
<td>Occipital (-5.8)</td>
<td>25.75 ± 4.78</td>
<td>34.05 ± 7.81</td>
<td>23.00 ± 8.61</td>
<td>32.37 ± 5.89</td>
</tr>
</tbody>
</table>

Data are mean ± SD. $^a$numbers in parentheses are the coordinates in mm from Bregma (Paxinos and Watson, 1997). DMI = desipramine hydrochloride 15 mg/kg i.p for 17d, VEH = vehicle (sterile water). $^b$Significant effect of treatment (p < 0.0009), (p < 0.0001), and significant treatment/region interaction (p < 0.007); $^c$significant effect of treatment (p < 0.007), region (p < 0.0001), and significant treatment/region interaction (p < 0.02).
Figure 3.7 shows graphical examination of the treatment/region interaction effect detected in the binding data from both radioligands. The frontal and parietal cortices had similar receptor binding in the vehicle treated animals, whereas prefrontal and occipital cortex binding were much higher and lower respectively.

**Comparison of PET and Tritiated Ligands**

Paired t-tests indicate no significant differences in 5-HT2 binding in the frontal and parietal cortices as measured by [3H]ketanserin and [18F]setoperone. However, binding in the prefrontal and occipital cortex was slightly, but significantly lower with [3H]ketanserin compared to [18F]setoperone (p < 0.0042 and p < 0.04 respectively). Figure 3.8 shows representative images obtained with [3H]ketanserin and [18F]setoperone at the level of the frontal cortex.

**Discussion:**

The main purpose of this study was to demonstrate and validate the use of phosphor imaging systems for quantitative receptor binding autoradiographic studies with tritiated and positron-emitting ligands. In particular, we provide three new pieces of information: 1) by correcting for differences in specific activity, and taking precautions in the analysis of standard curves to limit variability, autoradiographic phosphor imaging data collected with positron-emitting ligands made on different days is highly reproducible; 2) using tracers for the same receptor subtype, the data obtained from receptor autoradiographic phosphor imaging with positron-emitting ligands corresponds well to that obtained with the more traditional tritiated ligands; and 3) although tritium-sensitive phosphor screens lose their sensitivity over time, with post-fixation to limit contamination and background subtraction, they can be reused at least seven times, and for at least three months after the first exposure to obtain quantitative data.

This study has also confirmed the distribution of cortical 5-HT2 receptors. Autoradiographic studies with [3H]ketanserin indicate high 5-HT2 binding in layers III, IV and V of the cortex in a rostro-caudal gradient in humans, monkeys, and rats (Pazos et al., 1985; Hoyer et al., 1986; Lidow et al., 1989; Appel et al., 1990). The distribution of 5-HT2 binding in our study agrees closely with the literature. However, although the numerical binding values are comparable to some reports (Lidow et al., 1989; Appel et al., 1990), they are tenfold lower than the data from others (Pazos et al., 1985; Hoyer et al., 1986). The cause of this discrepancy is difficult to determine, as the only difference in incubation conditions in these studies is the drug used for non-specific binding. However, the close concurrence of our data with that of other studies in rat (Appel et al., 1990) validates the use of tritium-sensitive phosphor screens as an appropriate alternative to film.

Although we found essentially the same results with [3H]ketanserin and [18F]setoperone, the binding values for [3H]ketanserin were lower in both the DMI and vehicle treated animals in the prefrontal cortex (table 3.4). This may be a result of the post-fixation of the [3H]ketanserin bound slides in paraformaldehyde vapour, as slightly decreased binding and significantly decreased affinity (Kd) have been reported in the literature after this post-fixation protocol (Liberatore et al., 1999; Pavey et al., 2002). Alternatively, the discrepancy may result from differences in binding kinetics of the two ligands. In any case, the relative changes in 5-HT2 binding measured with [3H]ketanserin and [18F]setoperone are similar in the prefrontal cortex and in all other regions. The close concurrence of the data obtained with the two ligands validates the use of in vitro phosphor imaging with positron-emitting ligands to corroborate in vivo PET studies, instead of the more traditional, but also more time consuming, tritiated ligand autoradiography.
Figure 3.7: Decreased 5-HT\textsubscript{2} binding after chronic DMI treatment. Data are mean ± SD. There is a significant effect of treatment and region, and a significant interaction between the two, a reflection of the relatively high density of 5-HT\textsubscript{2} receptors in the prefrontal cortex, and relatively low density in the occipital cortex (see Discussion).
Figure 3.8: Autoradiographic detection of decreased 5-HT$_2$ binding using phosphor imaging and two different radioligands. A) $[^{18}\text{F}]$setoperone and B) $[^{3}\text{H}]$ketanserin binding in the frontal cortex in a DMI- (left) and vehicle-treated (right) animal. Sections were taken at approximately +1.7 mm from Bregma, according to Paxinos and Watson (1997).
Previous reports of decreased 5-HT\textsubscript{2} binding after chronic DMI treatment of rats are limited to either whole cortex or frontal cortex homogenates (Bergstrom and Kellar, 1979; Goodnough and Baker, 1994). However, because autoradiography has higher spatial resolution compared to binding studies using tissue homogenates, we were able to observe, for the first time, that chronic DMI treatment decreases binding to 5-HT\textsubscript{2} receptors throughout the cerebral cortex, and that this effect also follows a rostro-caudal gradient. In general, 5-HT\textsubscript{2} binding was decreased by chronic DMI treatment in rats to a greater extent in anterior cortical regions, confirming in vivo observations by PET in humans (Yatham et al., 1998). The region/treatment interaction (Fig. 3.7), resulted from major differences in the normal distribution of the receptors throughout the rostro-caudal extent of the cortex, so that the percent decrease in binding was greater in anterior regions of the cortex because the density of receptors is higher, and therefore the effect of chronic DMI treatment is more substantial.

Although we did not perform Scatchard analysis to determine whether changes in 5-HT\textsubscript{2} binding were due to altered affinity or density of the receptors, most reports indicate that chronic DMI treatment decreases the number of receptors, and not their affinity (Bergstrom and Kellar, 1979; Dumbrille-Ross et al., 1982; Cross and Horton, 1988). However, all previous investigations into the effects of chronic DMI on 5-HT\textsubscript{2} receptors have been performed on membrane homogenates. There are discrepancies in the literature between ligand binding data obtained in homogenates compared to slices, and in general, the affinity and/or density of the receptors is greater in tissue sections than in homogenate preparations (Dohanich et al., 1986; Revila et al., 2000; Pavey et al., 2002). This effect may be related to slice thickness and the subsequent slower equilibration rate with thicker slices, as apparent K\textsubscript{d} decreases with decreasing slice thickness (Dohanich et al., 1986), and must be determined for each ligand. In any case, quantitative receptor binding data obtained in homogenates and slices is probably not fully comparable. It is possible to perform Scatchard analysis using the phosphor imaging autoradiographic method with either tritiated or positron-emitting compounds (e.g. Gatley et al., 1998; Pavey et al., 2002, Nikolaus et al., 2003), and the higher spatial resolution of this technique compared to homogenate binding allows more precise localization of changes.

**Tritium-sensitive phosphor screens**

One of the limitations of phosphor imaging with tritiated ligands has been screen contamination, which leads to poor reusability and increased costs. To allow detection of the low energy radiation of tritium (maximum 18.6 keV, mean 5.7 keV) these screens do not have a protective coating. To reduce contamination, we have adopted the technique of Liberatore and colleagues (1999) and post-fix the slides in paraformaldehyde vapour in a vacuum for two days before apposition to the phosphor screens. We have also found that removal of any dirt or particulate matter on the glass slides (e.g. grease pencil used to contain the radioligand during incubation) before exposure prolongs the usable life of the tritium-sensitive phosphor screens. Since adopting these precautions, we have not observed any screen contamination in the form of latent (or "ghost") images identified in subsequent scans.

Another issue to consider is the loss of sensitivity of the tritium-sensitive screens over time. Without a protective coating, the bare phosphor crystals accumulate moisture, leading to increased background and reduced sensitivity. We tested the response of the screens over time, and found that the response of the screens remains linear, and that the loss of sensitivity due to accumulated moisture can easily be corrected by background subtraction up to three months after first opening. However, we still recommend that tritium-sensitive phosphor screens opened at or around the same time be used as a set for subsequent exposures.

We have found that with the post-fixation of the tissue, careful handling of the screens, and correction for loss of sensitivity over time, tritium-sensitive phosphor screens can be reused
to obtain quantitative data at least seven times, and that they retain a high level of sensitivity for at least three months. Our experience also suggests that if it remains uncontaminated, the useable life of a tritium-sensitive phosphor screen could be even longer.

**Phosphor Imaging with PET Ligands**

Quantitative phosphor imaging with tritiated ligands has been well described (Liberatore et al., 1999; Pavey et al., 2002). However, because of the short half-lives of positron-emitting ligands, quantification of phosphor autoradiography with PET tracers is difficult because standards have to be made daily. There are reports of the use of brain paste standards incubated with positron-emitting ligands (Nikolaus et al., 2001; 2003), but these are not always possible to create, especially when using ligands labelled with a short half-life nuclide such as $^{11}$C ($T_{1/2} = 20.4 \text{ min}$). We have adopted the technique of Gatley et al. (1998), the use of TLC plates with a set of serial dilutions of 5 µL drops of known activity. These standards are not only easy to produce, but also consistently and reproducibly provide linear standard curves with excellent fit. The correction for spillover that we describe above, whereby the image is displayed relative to the area in question, improves two characteristics of the standard curves. The goodness of fit of the curve is enhanced, and the slopes of the standard curves created on a single day and across days are more comparable, thereby decreasing the variability of receptor binding data from different screens and different days (Figs. 3.3 & 3.4). The spillover effect may also impact the receptor binding data directly. This could occur if, in the image, regions of particularly high binding are surrounded by regions of relatively low binding. As in the measurements of the points in the standard curve, we suggest that when placing ROIs, the investigator display the image relative to the area in question to minimize the effects of spillover.

The spatial resolution of phosphor imaging autoradiography is a function of three main factors: the optics of the imaging system, the characteristics of the phosphor screen, and the energy of the isotope used. Each phosphor imaging system produces an image with a specific pixel size, based on the properties of the scanning laser and associated electronics. According to the manufacturer, the Cyclone phosphor imager uses a laser that is focused to less than 50 µm, and scanning the screens at the highest resolution setting (600 dpi, as used in this report), displays an image with 42 µm pixels, only slightly larger than that of more expensive phosphor imaging systems (the Fuji BAS-5000 imaging system has a minimum image pixel size of 25 µm). In contrast, phosphor screens of all types have an intrinsic resolution of approximately 50 µm, based on the size of the photosensitive phosphor crystals (Yanai et al., 1992). This is fairly high resolution, but is still many times lower than film or emulsions, which can generally resolve on the order of a fraction of a micron. Finally, the energy of the isotope used to label the ligand contributes substantially to the resolution of the final images. Low energy particles (such as the β emission of tritium) interact only with the silver halide or phosphor crystals directly overlying them in the film or screen, giving superb resolution. In contrast, isotopes with higher energy, particularly the positron and gamma emission of positron-emitting ligands, are expected to have much lower resolution. Both our estimated resolution for $^{18}$F-phosphor imaging (470 µm) and our observation that the resolution was slightly better in the horizontal plane than vertically are similar to observations of the resolution of $^{11}$C-phosphor imaging by Sihver and colleagues (1997), who found FWHM values of 566 µm horizontally and 650 µm vertically. Sihver and colleagues (1997) attributed the horizontal/vertical resolution discrepancy to the mechanics of scanning the screen in a vertical direction, and our data support this hypothesis. We did not measure the resolution of the tritium-sensitive phosphor screens (for fear of long-lasting radioactive contamination of the Inkjet printer). In general, the spatial resolution achieved with phosphor imaging is well beyond that required for quantitative receptor autoradiography.
Due to the high cost of synthesizing the tracers, we perform in vitro studies with PET radioligands with the leftover doses from the patient scans. If there is not enough activity to perform the binding assay on all of the slides from one study, or there are too many slides to physically process in one day, the data may have to be collected over several days. Differences in specific activity lead to differences in the amount of radioactivity added to the incubation dishes in order to maintain a constant incubation concentration of the ligand, which must be corrected for by dividing the volume corrected data (nCi/cc of tissue) by the specific activity (Ci/mmol) to obtain a unit of pmol/cc. In this experiment, we have shown that even when the specific activities are widely different (265 Ci/mmol vs. 1214 Ci/mmol), correcting for the specific activity produces data that are statistically equivalent (table 3.3).

Chronic DMI treatment has dramatic effects on 5-HT2 binding, ranging from 24-38% decreases (tables 3.3 and 3.4). Changes that are less dramatic, but yet still biologically meaningful may be more difficult to detect by quantitative autoradiography with PET tracers. Power analysis indicates that due to the high variance of the data of some regions, the ability to detect a significant difference in the means of two groups as little as 10% with 80% power at the 0.05 level of significance, would require 30 or more animals per group. This number of subjects is not always feasible, and lack of sensitivity due to high interindividual variability is the major limitation of this technique. However, since we performed three separate binding studies, the amount of tissue we had available for each was limited, and binding measures were taken on only two sections per animal in each study. In the future, we recommend using four or more sections per animal to decrease the variability.

Multisensitive phosphor screens are quite versatile and can be used to perform autoradiographic studies with many different nuclides. We have found no loss of sensitivity in the screens over three years of use, and although very hot sources (in the mCi range) can leave behind latent ("ghost") images after scanning, these ghost images fade over time, and are generally lower than background noise in an $^{18}$F experiment (Strome et al., unpublished observations). Besides $^{18}$F-labelled radioligands, we have also performed quantitative receptor binding studies with $^{11}$C-labelled PET radioligands such as the D1 and D2 antagonists SCH 23390 and raclopride, and the vesicular monoamine transporter-2 ligand dihydrotetrabenazine (unpublished). Phosphor imaging is certainly not limited to autoradiography with PET or tritiated ligands. An especially common application of involves the use of $^{125}$I or $^{35}$S for quantitative receptor or in situ hybridization autoradiographic studies, (Key et al, 1991; Ito et al., 1995; Tang et al., 1995; Vizi et al., 2001). The energy of $^{35}$S is similar enough to $^{14}$C that commercially available $^{14}$C standards can be employed for quantification, while $^{125}$I microscales themselves are also commercially available. In fact, phosphor imaging systems can be used for almost any assay that involves detection of a 2-D radioactive source, making them a valuable addition to the array of standard laboratory equipment.

In conclusion, we have described several ways to exploit the inherently high resolution and sensitivity of storage phosphor screens to obtain quantitative receptor binding data that show good reliability and reproducibility with either tritiated or positron-emitting ligands. The temporal, economical, and practical advantages of phosphor imaging make it an excellent technique to replace traditional x-ray film tritiated ligand autoradiography. In particular, quantitative in vitro phosphor imaging with positron-emitting ligands provides an important tool to corroborate in vivo PET data with higher resolution in vitro autoradiographic data.
References:


Chapter 4

EVALUATION OF THE INTEGRITY OF THE DOPAMINE SYSTEM IN A RODENT MODEL OF PARKINSON’S DISEASE: SMALL ANIMAL PET COMPARED TO BEHAVIOURAL ASSESSMENT AND AUTORADIOGRAPHY

Preamble:
In the previous chapter we gave a detailed description of a method for using PET tracers for quantitative in vitro autoradiography. The technique was developed to provide us with a tool to use to address our specific hypotheses regarding the effects of repeated ECS treatment on DA receptors in 6-OHDA-lesioned rats. To address our hypotheses about the effects of repeated ECS treatment on the motor function of 6-OHDA-lesioned rats we decided to move away from the traditional drug-induced behavioural testing that is so commonly used in this animal model of PD (rotational behaviour after apomorphine or amphetamine treatment) because of the potential confounds that these DAergic drugs add to our primary neurochemical outcome measures. For example, even a single treatment with apomorphine sensitizes D1 receptors (Morelli et al., 1999), and apomorphine and amphetamine can induce GDNF, BDNF, and FGF-2 (Meredith et al., 2002; Guo et al., 2002; Mueller et al., 2006). Instead, we adopted two non-pharmacological behavioural tests; the Cylinder Test, which measures forelimb function, has been shown to correlate with striatal DA levels (Tillerson et al., 2001), and is widely used to evaluate therapeutic interventions in the unilateral 6-OHDA-lesion rat model of PD (Cohen et al., 2003; Shi et al., 2004), and the tapered/ledged beam-walking (TB) test (Schallert and Woodlee, 2005), which has been less thoroughly investigated. In this chapter, we examined the relationship between performance on the TB test and striatal DA terminal integrity in animals with mild or severe unilateral 6-OHDA lesions, as measured by our in vitro autoradiographic technique described in Chapter 3, and by in vivo microPET imaging, using the small animal PET scanner that we had gained access to in the meantime. These PET studies also allowed us to investigate the possibility of using microPET to investigate our primary hypotheses regarding the mechanism of action of ECT in PD. In the end, we decided against using microPET for our preliminary studies for several reasons, including the poor resolution of the technique, and the difficulty in gaining access to the scanner and required tracers at specific time-points before, during, and after the course of ECS treatment.

Introduction:
In recent years, our understanding of the role that genes and/or the environment play in disease, and the development of therapeutic options have greatly improved as a result of better animal models, achieved through advances in genetic engineering or the use of specific toxins. Traditionally, behavioural analysis, with or without pharmacological challenge, was the method of choice for non-invasive longitudinal assessment of animal models of disease. There are several problems with relying solely on behavioural observation to evaluate animal models, however, including the fact that it does not provide specific information about underlying biochemical processes, and individual variation may be very large. A classical example of the evaluation of an animal model heavily dependent on behavioural analysis is the unilateral 6-

---

1 A version of this chapter has been accepted for publication. Strome EM, Cepeda I, Sossi V, Doudet DJ (2006) Evaluation of the integrity of the dopamine system in a rodent model of Parkinson's disease: small animal PET compared to behavioral assessment and autoradiography. Molecular Imaging and Biology.
hydroxydopamine (6-OHDA, a selective neurotoxin of the nigrostriatal pathway) lesion rat model of Parkinson’s disease (PD; Ungerstedt, 1968). In the past, longitudinal assessment of this model depended primarily on repeated pharmacological challenges (Costall et al., 1976), which in themselves can modify the system. The best of example of this is the robust observation of sensitization of both the behavioural response and the D1 receptor after repeated apomorphine (Morelli et al., 1989; Klug and Norman, 1993). The 6-OHDA rat model is therefore a good example of an animal model of a human disease that can greatly benefit from advances in our ability to reliably, reproducibly, and sensitively evaluate longitudinally, in vivo, the disease process or the effects of therapeutics.

Positron emission tomography (PET) is a powerful tool for in vivo imaging of biochemical processes. The low spatial resolution of the technique (maximum ~ 4 mm), however, necessitates the use of large animals as subjects (humans and non-human primates). Unfortunately, most biomedical research is done using small animals, particularly rodents, birds, fish or amphibians. In response, small animal PET (microPET) is an emerging field, and scanners are now commercially available with smaller fields of view, sensitive scintillation detectors, and higher spatial resolution (Tai et al., 2001; Knoess et al., 2003; Tai et al., 2003; Yang et al., 2004; Tai et al., 2005). These microPET scanners show great promise for use in life sciences research, particularly in the fields of drug development, oncology and cardiology. Although still fairly expensive and requiring the typical infrastructure of a full-size PET program, microPET offers many advantages over traditional post-mortem analysis in animal research, including limiting the number of animals used (by allowing longitudinal studies in the same animal, particularly important in studies of genetically modified mice), and direct translation to the clinic (through the use of tracers that can be directly applied to patients in clinical PET settings). Nevertheless, few studies have rigorously evaluated the performance of in vivo microPET compared to traditional instruments such as behaviour or post-mortem measures.

In this study, we endeavoured to compare estimates of the severity of the lesion induced by intracerebral 6-OHDA using two in vivo measures, behaviour and microPET imaging, and one in vitro measure, autoradiography. The first challenge was to decide which PET tracer to use because changes in the availability of synaptic dopamine (DA) often lead to compensatory responses by pre- and/or post-synaptic aspects of DA neurotransmission. For example, DA receptors can be upregulated by the application of DA antagonists (Huang et al., 1997) or DA terminal degeneration as in PD (Rinne et al., 1995) or experimental lesion models (Narang and Wamsley, 1995; Nikolaus et al., 2001; Doudet et al., 2002), while the DA transporter (DAT) is upregulated by chronic cocaine administration (Wilson et al., 1994), but downregulated by pharmacological DA depletion (Kilbourn et al., 1992) or in early parkinsonism (Lee et al., 2000; Doudet, 2001). The vesicular monoamine transporter (VMAT2, which packages dopamine into vesicles), however, appears to be insensitive to changes in synaptic DA, and compensatory responses to DA enhancing and depleting stimuli generally do not occur (Vander Borght et al., 1995; Wilson and Kish, 1996). The VMAT2 is therefore widely regarded as a gold standard for measuring the integrity of striatal DA terminals.

The drug most widely used to evaluate the VMAT2 is tetrabenazine and its derivatives, methoxytetrabenazine and dihydrotetrabenazine (DTBZ). Both of the latter two have been radiolabelled with [3H] for in vitro binding studies and with [11C] for in vivo PET studies (Frey et al., 1998). Thus, in this pilot validation study, we selected [11C](±)dihydrotetrabenazine ([11C]DTBZ), and compared in vivo data from a microPET scanner to both in vitro autoradiography with the same tracer, and to behavioural analysis after a mild or severe unilateral 6-OHDA lesion of the nigrostriatal pathway.
Materials and Methods:

Subjects

Adult male Sprague-Dawley rats (n = 13, 300-350 g at the time of lesion) were housed in dyads with food and water available ad libitum. Their housing room was kept at constant humidity (55%) and temperature (21°C), and had a 12:12 light:dark cycle, with lights off at 12:00 p.m.

6-OHDA Lesioning

All animals were given desipramine hydrochloride (Sigma, 25 mg/kg i.p.) 30-60 min before 6-OHDA infusion to protect noradrenergic terminals. Animals were anaesthetized with isoflurane in O2 (4% for induction, 1% for maintenance), given atropine sulfate (0.05 mg/kg s.c.), and placed into a stereotaxic head holder (Kopf). Two different lesion protocols were used, meant to create either a severe or a mild unilateral lesion. The animals received either a total of 20 µg of 6-OHDA hydrobromide (Sigma; n = 7, rats A-G) delivered in 2 sites along the medial forebrain bundle (10 µg in 4 µl of saline plus 0.05% ascorbic acid per site; Coordinates: site 1: AP -2.8 mm, ML -1.8 mm, DV -8.0 mm (all from Bregma); site 2: AP -4.7 mm (Bregma), ML -1.5 mm (midline), DV -7.9 mm (hole), according to (Paxinos and Watson, 1997)) or 2 µg of 6-OHDA at a single site along the MFB (the 2nd infusion site listed above; n = 6, rats H - M). The infusion rate was 1 µL per min, and the cannula was left in place an additional 4 min to allow diffusion of the 6-OHDA solution. After surgery, the animals received subcutaneous saline, antibiotics (Duplocillin 0.1 ml/kg i.m.), and analgesia (Anafen 2 mg/kg s.c.), and were kept warm in an incubator until fully recovered from anaesthesia. The animals were allowed two weeks to recover from surgery, and their weight and condition were monitored daily during the first week of recovery. Two of the study animals in the severe lesion group were recruited from a different study, where they were excluded after lesioning based on their behavioural findings (few errors made with the hindlimb contralateral to the lesion on the tapered/ledged beam-walking test – see below).

Tapered/Ledged Beam-Walking (TB) Test

Prior to lesioning, animals were trained on a tapered/ledged beam-walking (TB) test, adapted with slight modifications from Schallert and colleagues (Schallert and Woodlee, 2005; Zhao et al., 2005). This test is similar to traditional rat beam-walking tasks most commonly used in models of stroke, but also sensitive to DA function (Feeney et al., 1982; Walsh and Wagner, 1992; Bowenkamp et al., 2000). The TB test apparatus is shown in Figure 4.1. This task requires the animals to walk along a 165 cm long, progressively narrowing (6.5 cm wide at the wide end, 1.5 cm at the narrow end) Plexiglas beam, elevated above the floor on an incline of 15 degrees, to reach their darkened home cage. The main surface of the beam is covered in rubber matting to provide traction. Two cm below the beam, there is a 2.5 cm wide Plexiglas ledge, which provides a platform to step on when there is a motor deficit. It is this ledge that makes this particular beam-walking test unique, in that the ledge allows the animals to express their motor deficit, and removes the need for postural compensation to prevent falling off the beam (Schallert and Woodlee, 2005). Taking a step with only 1 or 2 toes on the main surface of the beam (and the other 4 or 3 toes overhanging the ledge) is scored as a half footfault, while stepping with the entire foot on the ledge rather than on the main surface of the beam is scored as a full footfault. Training on the TB test involves 10 trials, with the animal starting further and further away from the home cage goal (3 trials starting at the beginning of the narrow end, 3 starting at the beginning of the medium end, and 4 starting at the wide end). In between trials, the animal remains in its home cage with the lights in the room turned off for one minute for reinforcement. The animals learn this task quickly, and training need only occur once in the
lifetime of the animal. Normal animals make very few errors (footfaults), and those only occur on the narrowest section of the beam.

![Tapered/ledged beam-walking (TB) test apparatus.](image)

Figure 4.1: The tapered/ledged beam-walking (TB) test apparatus.

All animals were trained prior to lesioning and tested 2-3 weeks post-lesion, a period which corresponded to 1-2 weeks prior to the microPET scan. On the testing day, the animals were brought to the behavioural room during the dark cycle and allowed to habituate for 15 - 30 min. Before testing, each animal is allowed one “refresher” trial, which is not videotaped. One TB test is made up of 5 consecutive trials, and each trial was videotaped from the rear to allow a clear observation of the hindlimbs, and scored at a later date by an investigator blind to the animal’s lesion condition. For each hindlimb, the number of steps taken in each of the three bins (wide, medium and narrow portions of the beam) and the number of full and half footfaults in the 5 trials was determined. Because the scores represent mainly large or small proportions (# errors per steps), transforming the data via an arcsine transformation (Eq. 4.1) normalizes the data (Zar, 1999).

\[
\text{Equation 4.1: } p' = \frac{1}{2}[(\arcsin \sqrt{X/n + 1}) + (\arcsin \sqrt{X + 1/n + 1})]
\]

Where X = # errors, and n = # steps.

**MicroPET Imaging**

On the day of the PET scan, the animal was brought to the scanning room and anaesthetized with ketamine:xylocaine (90:10 mg/kg i.m.), and given atropine sulfate (0.05 mg/kg s.c.). After the induction of anaesthesia, the animal was positioned prone in a plastic head holder attached to the bed of the scanner (Rubins et al., 2001) to ensure that all of the study animals were positioned with a flat skull and in the same plane, and an i.v. line was placed in a lateral tail vein. A transmission scan with a rotating Ge-68 point source supplied by the scanner
manufacturer was performed prior to radiotracer injection and the data were used for attenuation correction. The tracer \[^{11}\text{C}]\text{DTBZ}\) was synthesized as described previously (Jewett et al., 1997). Animals were injected with 0.1 mCi/100g body weight \[^{11}\text{C}]\text{DTBZ}\) (278-2738 Ci/mmol at injection; median 1603 Ci/mmol), and scanning with a microPET R4 scanner (Concorde Microsystems, Knoxville TN) occurred for the 30-60 min post-injection. Data were reconstructed by filter back projection after attenuation and scatter correction (Alexoff et al., 2003), and the images from the 16 time frames were summed to produce a higher statistic image of the rat brain. Regions of interest (ROI) analysis was performed using the system’s software (ASIPro 6.0). Small, circular ROIs (area = 6 mm²) were placed bilaterally on the 3 slices containing striatum, and oval ROIs (area = 20 mm²) were placed in two slices containing cerebellum. Binding data are presented as the average of the left or right striatal ROI values divided by the average of the cerebellar ROI values, minus one, to give an estimate of the left or right striatal binding potential (BP).

**Autoradiography**
After fully recovering from the anaesthesia used for the microPET scan, the animals were sacrificed by decapitation, and the brain quickly removed and frozen in isopentane cooled to -70°C with dry ice. The brains were stored in a freezer at -80°C until sectioning on a cryostat (Leica) at -17°C. Twenty micron sections were taken through the striatum and thaw mounted onto glass slides (Superfrost Plus, Fisher Scientific). The tissue was then stored in the -80°C freezer until autoradiography was performed.

The details of the quantitative autoradiographic procedure have been described in detail (Strome et al., 2005). Specifically, for \[^{11}\text{C}]\text{DTBZ}\) binding, the buffers were taken from (Vander Borght et al., 1995). The slides were removed from the freezer and allowed to warm up to room temperature. Following pre-washing for 5 min at 25°C in sucrose buffer (300 mM sucrose, 50 mM Tris-HCl, 1 mM EDTA, pH 8.0), the slides were incubated for 30 min in the same buffer with the addition of 5 nM \[^{11}\text{C}]\text{DTBZ}\). Non-specific binding was performed simultaneously on an adjacent set of sections with the addition of 1 μM unlabelled tetrabenazine. After incubation, the slides were washed twice for 3 min each in the same sucrose buffer at 25°C, and then briefly dipped in deionized distilled water at 4°C to remove excess sucrose. The slides were then allowed to dry for 20 min in a fume hood, and then placed against multisensitive storage phosphor screens (Perkin-Elmer) along with a set of standards of known activity (Strome et al., 2005) in standard film cassettes. The cassettes were stored behind lead for the 2 h exposure time, at which point, the screens were removed and scanned in a Cyclone phosphor imaging system (Perkin-Elmer) at 600 dpi. The binding data were analyzed as described (Strome et al., 2005) using the Cyclone’s inherent software (Optiquant v.4.0). Small circular ROIs (area = 5 mm²) were placed in the striatum. Total binding data were averaged over 6 sections per animal from at least 3 levels of the striatum (anterior, mid and posterior, chosen to duplicate the three striatal frames on which ROIs were placed in the microPET data), and non-specific binding was subtracted. The data were converted from DLU (digital light units) to pmol/cc tissue using the standard curves and the specific activity of the tracer at incubation (Strome et al., 2005).

**Statistical Analysis**
Because the animals primarily made mistakes in the narrow section of the beam, each animal’s TB test score was calculated based on the number of errors made per steps taken on the narrow section of the beam in all 5 trials with both hindlimbs. The distribution of the autoradiographic, microPET and behavioural data was tested for normality (Kolmogorov-
Smironov test), and Pearson product moment correlation analysis was performed with GraphPad Prism v.3.0 for Windows (San Diego, CA).

**Results:**

TB test data were not obtained for 2 animals (animals E and H in Table 1 and Fig. 4.3) due to a technical mishap (footage accidentally taped over).

Figure 4.2 shows correlation analyses of the three measures of striatal DA integrity. There was a significant correlation between in vivo and in vitro measures of striatal \(^{[1]}\text{C}\)DTBZ binding (Pearson product-moment correlation, \(r = 0.64\) p < 0.005; Fig. 4.2a). There was also a significant correlation between hindlimb errors on the narrow section of the TB test and striatal \(^{[1]}\text{C}\)DTBZ binding as measured in vitro with autoradiography (\(r = -0.43\), p < 0.05; Fig. 4.2b). TB test scores were not significantly correlated with striatal \(^{[1]}\text{C}\)DTBZ binding as measured in vivo with microPET (\(r = -0.05\), p > 0.05; Fig. 4.2c).

Table 4.1 shows the lesion severity as measured in vivo by PET and at post-mortem with autoradiography. Five out of seven that received the high dose of 6-OHDA (animals A-E) showed severe unilateral depletion of striatal \(^{[1]}\text{C}\)DTBZ binding as measured with autoradiography. The remaining two animals in this lesion protocol (animals E-F) did not show any marked asymmetry in striatal \(^{[1]}\text{C}\)DTBZ binding as measured with autoradiography, and, in fact, were the two who had been recruited from a different study in our laboratory where they had been excluded for having mild lesions based on a lack of errors with the hindlimb contralateral to the lesion on the TB test. Animals that received a low dose of 6-OHDA experienced mild unilateral 6-OHDA lesions, ranging from 30-65% according to the autoradiographic results. Similarly, these animals displayed little or no behavioural impairments (Fig. 4.3). Table 1 also shows that lesion severity measured with PET is much lower than lesion severity measured with autoradiography in the five severely lesioned animals (A-E), whereas in the animals with milder lesions, while lesion severity was generally lower when measured by PET, the two techniques gave much more comparable results.

Figure 4.3 shows TB test scores for both hindlimbs in the narrow section of the beam. Only animals that had severe unilateral 6-OHDA lesions as measured by autoradiography (> 90% depletion of \(^{[1]}\text{C}\)DTBZ binding in the lesioned striatum), the gold standard for measuring striatal DA terminal integrity after 6-OHDA lesion, also showed a severe impairment in their hindlimb contralateral to the lesion on the TB test (animals A-D; Fig. 4.3).

Figure 4.4 shows examples of microPET and autoradiographic images of striatal \(^{[1]}\text{C}\)DTBZ binding in three representative animals, showing A) an intact animal, B) an animal with a mild unilateral lesion, and C) an animal with a severe unilateral lesion. In the intact striatum, in vitro \(^{[1]}\text{C}\)DTBZ binding was 176.50 ± 14.31 pmol/cc (mean ± SD), and in vivo, the mean BP was 2.07 ± 0.68.
Table 4.1: Comparison of percent lesion after unilateral infusion of 20 μg (High Dose) or 2 μg (Low Dose) 6-OHDA, as measured by striatal $[^{11}]$C$DTBZ$ binding in vivo by microPET and in vitro with autoradiography.

<table>
<thead>
<tr>
<th>Animal ID</th>
<th>% Lesion in vivo$^a$</th>
<th>% Lesion in vitro$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Dose 6-OHDA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>60.84</td>
<td>92.59</td>
</tr>
<tr>
<td>B</td>
<td>59.17</td>
<td>98.40</td>
</tr>
<tr>
<td>C</td>
<td>49.67</td>
<td>96.49</td>
</tr>
<tr>
<td>D</td>
<td>41.73</td>
<td>92.92</td>
</tr>
<tr>
<td>E</td>
<td>58.23</td>
<td>91.94</td>
</tr>
<tr>
<td>F</td>
<td>47.06</td>
<td>44.07</td>
</tr>
<tr>
<td>G</td>
<td>62.90</td>
<td>74.42</td>
</tr>
<tr>
<td>Low Dose 6-OHDA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>26.36</td>
<td>29.92</td>
</tr>
<tr>
<td>I</td>
<td>40.94</td>
<td>56.25</td>
</tr>
<tr>
<td>J</td>
<td>36.36</td>
<td>30.76</td>
</tr>
<tr>
<td>K</td>
<td>31.82</td>
<td>45.82</td>
</tr>
<tr>
<td>L</td>
<td>36.00</td>
<td>64.49</td>
</tr>
<tr>
<td>M</td>
<td>30.12</td>
<td>30.80</td>
</tr>
</tbody>
</table>

$^a$ (100 - % ratio $[^{11}]$C$DTBZ$ BP lesioned/non-lesioned striatum)

$^b$ (100 - % ratio $[^{11}]$C$DTBZ$ binding lesioned/non-lesioned striatum)
Figure 4.2: Relationships between the three measures of striatal DA integrity. A) There is a significant correlation between autoradiography and microPET. B) There is a significant negative correlation between autoradiography and the TB test. C) There is no correlation between microPET and the TB test.
Figure 4.3: TB test scores for each hindlimb in individual animals after severe (A-G) or mild (H-M) unilateral 6-OHDA lesioning. Only data from the narrow section of the beam are shown. Black bars represent the hindlimb contralateral to the lesion.
Figure 4.4: Coronal images of striatal $[^{11}C]DTBZ$ binding through the head and brain with microPET (left) and in brain only with autoradiography (right). A) A normal control animal for comparison. B) An animal with a mild right unilateral lesion. C) An animal with a severe right unilateral lesion. For the purposes of publication, microPET images were maximum a posteriori (MAP) reconstructed.
Discussion:

The results of this study show that there is a strong correspondence between striatal DA terminal integrity as measured in vivo by microPET and in vitro by autoradiography using the same tracer, [11C]DTBZ. In addition, TB test scores were significantly correlated with our autoradiographic determination of striatal DA terminal integrity, but were not significantly correlated with striatal DA terminal integrity as measured by microPET. The TB test appears to be a good behavioural measure of DA-dependent motor function only in severely unilateral 6-OHDA-lesioned rats, suggesting that a lack of impairment on this task could be used as an exclusion criterion for animals with poor lesions prior to further interventions.

Our in vitro autoradiographic data are in good agreement with literature values of DTBZ binding in the intact and 6-OHDA-lesioned rat striatum. Although this is the first autoradiographic study with [11C]DTBZ, [3H]DTBZ has been widely employed to study the VMAT2, and our quantitative data correspond well to previous studies (Darchen et al., 1989; Masuo et al., 1990; Vander Borght et al., 1995).

Our data are also in good agreement with previous studies showing a strong positive correlation between small animal PET imaging of the brain and autoradiographic analysis with the same tracer. However, whereas previously it has been shown that this is the case for ex vivo autoradiography with the D2 receptor ligand [18F](N-methyl)benperidol (Nikolaus et al., 2003) and 2-[18F]fluoro-2-deoxy-D-glucose (Moore et al., 2000; Matsumura et al., 2003), our data are the first to show the strong correspondence with a C-11 labeled tracer and in vitro autoradiography in the same animal.

It is notable that although the correlation between [11C]DTBZ binding in the same animals in vivo compared to in vitro is very high, as shown in Fig. 2a, this correlation is not a perfect 1 to 1 relationship and the slope has a positive intercept (i.e. it does not go through the origin). This phenomenon arises from an overestimation of the low BP values in the lesioned striatum with microPET, and may have several explanations, including the existence of anaesthesia-related effects in the in vivo studies, and/or a bias in the in vivo data due to the intrinsic properties of the scanner, in particular, the limited resolution.

We chose to perform our microPET studies under ketamine/xylazine anaesthesia. Although ketamine, a non-competitive NMDA antagonist, is known to affect the binding of other DAergic radiotracers, namely those for D1 (Momosaki et al., 2004) and D2 (Kobayashi et al., 1995; Vollenweider et al., 2000) receptors, it is not known how ketamine or ketamine/xylazine may influence the binding of [11C]DTBZ to the VMAT2. An ongoing study of the effects of anaesthesia on microPET data in our lab indicates that, while, in comparison to isoflurane anaesthesia, ketamine/xylazine substantially decreases [11C]DTBZ binding in vivo, the effect is uniform in the striatum and cerebellum, and does not influence the striatum/cerebellum ratio (unpublished observations). At this point, we cannot exclude the possibility that ketamine/xylazine (or any anaesthesia) may have a differential influence on the binding of [11C]DTBZ in the lesioned vs. intact striatum. If the binding was affected to a greater extent in the intact striatum, this would decrease the apparent lesion severity, a phenomenon observed in this study, but which we attribute mainly to the partial volume effect (see next paragraph).

As shown in Table 1, while we were able to detect severe unilateral lesions autoradiographically, we were unable to detect large asymmetries in striatal [11C]DTBZ binding with microPET. In animals with severe unilateral lesions (>90%) as measured by autoradiography, microPET showed only 42-61% lesions, whereas in the animals that did not suffer severe lesions, the striatal asymmetry in [11C]DTBZ binding is comparable when measured either in vivo or in vitro. The inability to measure large asymmetries in striatal [11C]DTBZ binding in vivo in unilateral 6-OHDA lesioned rats most likely results from the
partial volume effect. Because the size of the rat striatum (width ~2-3 mm; (Paxinos and Watson, 1997) is generally less than twice the resolution of the scanner (~ 2 mm FWHM at the center of the field of view in each direction), it is susceptible to partial volume effects (Mazziotta et al., 1981). The partial volume effect would be particularly strong in the lesioned striatum, due to spillover from the adjacent intact striatum, which would lead to an overestimation of the true radioactivity concentration in the lesioned striatum. The partial volume effect, therefore, apparently decreases the quantitative accuracy of the microPET determination of $[^{11}\text{C}]$DTBZ binding in the lesioned striatum, suggesting that post-mortem measures should be included in any study examining unilateral striatal lesions and microPET imaging. More recent, better resolution scanners, as well as the use of other reconstruction algorithms allowing higher resolution and the use of co-registration of microPET with CT or MRI may also improve the identification of specific brain regions and ROI placement, and thus reduce the apparent effect of partial voluming. In addition to the partial volume effect, the microPET data in this experiment may also be subject to a “floor effect”, i.e. low sensitivity of the technique in measuring low striatal $[^{11}\text{C}]$DTBZ binding. We are currently performing a number of follow-up studies to further investigate some of these issues.

The TB test appears to be most sensitive in animals that have severe unilateral lesions, as these were the only animals in our study to show strong asymmetries between TB test scores with the hindlimbs ipsilateral and contralateral to the lesion (animals A-E; Fig. 3). Two of the animals in this study (animals F and G) had originally been assigned to another study, but were excluded based on their TB test scores (few errors made with the hindlimb contralateral to the lesion), and transferred to the current investigation to assess the status of the DA nigrostriatal system. Not surprisingly, these two animals also showed poor lesions when evaluated with both microPET and autoradiography. Indeed, none of the animals that had mild lesions according to our autoradiographic results showed a clear deficit with the hindlimb contralateral to the lesion on the TB task, and, in fact, some animals made more errors with the hindlimb ipsilateral to the lesion (Fig. 3). Since the severe unilateral 6-OHDA-lesion model is the most commonly employed rat model of PD, our data suggests that the TB test can be used to screen animals with poor lesions and remove them from further analysis early in a study, thereby reducing the waste of time, energy, and materials that occurs when an animal is excluded at the end of a study due to a poor lesion.

The same inherent issues with quantification of microPET data in unilateral 6-OHDA-lesioned rats discussed above may have contributed to the fact that TB test scores and $[^{11}\text{C}]$DTBZ binding measured with microPET were not significantly correlated, even though the autoradiographic results did correspond well to the TB test scores. Taken together, the limitations of microPET in measuring strong asymmetries in striatal DA innervation, and of the TB test in measuring mild asymmetries in striatal DA innervation, likely led to a poor correlation between these two measures.

Many beam-walking tasks designed to evaluate motor function have been reported in the literature, but the task that we report here is unique in its design. The ledge positioned below allows the animal to make slips off the main surface of the beam without a fear of falling, and thus allows them to express any motor deficit without masking it with postural compensations (Schallert and Woodlee, 2005). We have found this task to be useful in evaluating motor deficits in both severely unilaterally and bilaterally 6-OHDA-lesioned rats, and have been able to detect significant improvement in lesioned animals after anti-parkinsonian interventions (unpublished). Non-pharmacological behavioural testing in models of PD is an emerging trend, as evidenced by the popularity of many new tasks including the Forelimb Use Asymmetry Test (“Cylinder Test”), the Forelimb Placing Task, and the Sensorimotor Asymmetry Test (“Sticker Test”) (Schallert et al., 2000; Schallert and Woodlee, 2005), and the TB test is a valuable addition to the
battery of tools available to evaluate motor behaviour without the use of drugs, thereby removing an additional potential confound.

In conclusion, we have shown in this study, that while measurement of the integrity of the striatal DA system using microPET correlates significantly with in vitro autoradiography with the same tracer, autoradiography is more sensitive at detecting lesion severity, likely due to the substantial partial volume effect that can occur when imaging with the microPET R4 in the unilaterally 6-OHDA-lesioned striatum. Autoradiographic measurement of striatal \[^{11}\text{C}]\text{DTBZ}\) binding was also significantly correlated with behavioural measures of DA-dependent motor function. This study highlights the limitations of both behavioural testing and microPET imaging in the unilateral 6-OHDA-lesion rat model of PD, and emphasizes the strengths of autoradiographic analysis of striatal DA innervation. We also suggest that the TB test can be used in studies employing the severe unilateral 6-OHDA-lesion model as a means of quickly and easily evaluating lesion severity to exclude animals with poor lesions prior to further intervention.
References:


Chapter 5

ELECTROCONVULSIVE SHOCK ENHANCES STRIATAL D₁ AND D₃ RECEPTOR BINDING AND IMPROVES MOTOR PERFORMANCE IN 6-OHDA-LESIONED RATS

Preamble:

The previous three chapters of this thesis describe our preliminary studies evaluating both the animal models and the tools that we wanted to use to address our primary hypotheses regarding the mechanism of action of ECT in PD. These studies gave us a great deal of insight into the most appropriate methods and experimental design for the investigation of our main research question. First of all, it became clear that, although non-human primates would be a better model in which to investigate the effects of ECT in PD, their use was not financially, technically, or ethically feasible. For our goals, a rat model had many advantages, including the fact that the 6-OHDA rat model of PD is well-characterized, easily produced, and reproducible. Secondly, although the use of microPET imaging allows longitudinal studies to be performed, the difficulty of obtaining all the required tracers at specific time-points, as well as the poor resolution, precluded its consistent use for these preliminary studies. In addition, even though several groups are actively working at developing specific tracers for D₃ receptors, none is currently available for PET. Quantitative autoradiography, therefore, emerged as the technique of choice to investigate our hypotheses regarding ECS effects on DA receptors, and we have demonstrated several uses of the technique in these preliminary studies. Finally, several non-pharmacological behavioural tests have been described for the evaluation of motor deficits in the unilateral 6-OHDA rat model of PD that both allow simple evaluation of lesion severity, and are sensitive to therapeutic interventions. In the previous chapter, we evaluated the use of the TB test for characterizing hindlimb motor deficits in the unilateral 6-OHDA model, and showed that impairment on the task correlates with the degree of striatal DA terminal integrity as measured by autoradiography. Not included in the chapters, but reported in Appendix A, we also performed a small validation and feasibility study on the use of anaesthesia for rodent ECS, in an effort to design a rodent study as clinically relevant as possible. The outcome of this study led us to choose ketamine as the anaesthetic of choice for all future studies, based on its ease of use and relevance to the clinical situation, as well as the fact that we were able to reproduce the increase in 5-HT₂ receptor binding widely reported after treating unanaesthetized rodents with ECS.

At this point, we were ready to undertake our first investigations of the effects of repeated ECS treatment in a rat model of PD. This chapter describes our first study to directly address the working hypothesis (Fig. 1.1, page 10), and in it we examine Hypothesis 1, by evaluating forelimb and hindlimb motor function, as measured by the Cylinder and TB tests respectively, and Hypothesis 2, by measuring DA receptor binding using quantitative phosphor imaging autoradiography.

---

1 A version of this chapter has been submitted for publication. Strome EM, Zis AP, Doudet DJ (2006) Electroconvulsive shock enhances striatal D₁ and D₃ receptor binding and improves motor performance in 6-OHDA-lesioned rats.
Introduction:
Parkinson's disease (PD) is a neurodegenerative movement disorder characterized by tremor, bradykinesia, rigidity, and postural disturbances. The primary pathological features of PD are the loss of dopamine (DA)-producing cell bodies within the substantia nigra pars compacta of the midbrain, and the concomitant loss of striatal DA (Bernheimer et al., 1973). Therapeutic options for PD attempt to either replace the lost DA tone, for example by treating patients with L-DOPA (the biochemical precursor to DA) and/or DA agonists, or to inhibit the basal ganglia output structures that become overactive in PD via surgical intervention, as in the case of pallidotomy or deep brain stimulation (DBS). Most patients, however, experience negative side effects or a loss of efficacy after prolonged L-DOPA treatment (reviewed in Jankovic, 2005), and not all patients are good candidates for brain surgery. The development of adjunctive or alternative therapeutic options for PD is essential in order to improve the quality of life of patients living with this disease.

Electroconvulsive therapy (ECT) is a widely used treatment for psychiatric disorders, and several recent meta-analyses have shown that it is the most effective antidepressant treatment available (Kho et al., 2003; Pagnin et al., 2004). Not only is ECT an effective treatment for depression, but it also appears to have positive effects on the motor symptoms of PD patients, regardless of whether or not they are depressed. Over 200 PD patients treated with ECT have been reported in the literature (Kennedy et al., 2003), with the majority showing dramatic improvement in their motor symptoms. Case reports, open trials, and double-blind placebo-controlled studies have all shown that ECT treatment significantly improves a wide range of motor symptoms, including rigidity, bradykinesia, and "on-off" phenomenon, and the improvements last from several weeks to months after the last treatment (Andersen et al., 1987; Stern, 1991; Zervas and Fink, 1991; Fall et al., 1995; Pridmore and Pollard, 1996). While there is still a need for further, well-designed clinical trials, with large numbers of subjects to determine the most appropriate parameters needed to achieve optimal impact on the motor symptoms of PD, this treatment holds great promise as a potential adjunct treatment.

In animals, repeated treatment with electroconvulsive shock (ECS) has been shown to have specific effects in limbic brain regions such as the frontal cortex and hippocampus. One of the most consistent effects of repeated ECS on the normal rodent limbic system is an enhancement of serotonin (5-HT) neurotransmission, as evidenced by increased 5-HT-mediated behaviours (Green et al., 1983a; Goodwin et al., 1984; Moorman et al., 1996), increased interstitial 5-HT metabolites (Yoshida et al., 1998), and upregulation of the 5-HT2 receptor (Green et al., 1983b; Butler et al., 1993). Taken together with increases in neurotrophic factors (Nibuya et al., 1995; Angelucci et al., 2002) and cell growth (Vaidya et al., 1999; Malberg et al., 2000), the enhancement of 5-HT neurotransmission after repeated ECS is part of a cascade of cellular events thought to underlie the mechanism of action of ECT in mood disorders (Duman et al., 1997).

We suggest that the effects of repeated ECS treatment on the DA system of the parkinsonian striatum may be similar to those observed on the 5-HT system of the hippocampus, and that these changes may partially underlie the positive effects of ECT on the motor symptoms of PD. In this study, we hypothesize that repeated ECS treatment in unilateral 6-hydroxydopamine (6-OHDA)-lesioned rats, a rat model of PD, will improve motor function and increase binding to DA receptors of the direct pathway of the basal ganglia.
Materials and Methods:

Subjects

Adult male Sprague-Dawley rats [bred at the University of British Columbia animal facility from Charles River Canada (Montreal, PQ) stock], weighing 250 g at the start of the experiment were housed on a 12:12 light:dark schedule (with lights off at 12:00 p.m.), at constant temperature and humidity (21°C, 55%). The animals had access to food and water ad libitum, and were housed in pairs. Twenty of the animals were used for D₁ receptor binding (n = 10 sham and 10 ECS) and 20 were used for D₂ and D₃ receptor binding. Cylinder Test data were pooled from the entire group of animals, while data for the TB test also include data from animals in a parallel study (Strome et al., 2006b). All procedures were approved by the University of British Columbia Committee on Animal Care.

6-OHDA Lesioning

The animals were allowed to habituate and were handled for at least three days before receiving a right unilateral 6-OHDA-induced lesion of the DA nigrostriatal pathway. Desipramine hydrochloride (25 mg/kg i.p.; Sigma-Aldrich Canada, Oakville, ON) was administered 30-60 min before 6-OHDA infusion to protect noradrenergic terminals. Animals were anaesthetized with isoflurane in O₂ (4% for induction, 1% for maintenance), given atropine sulfate (0.05 mg/kg s.e), and placed into a stereotaxic frame (Kopf). With the skull flat between lambda and Bregma, a 2% solution of 6-OHDA hydrobromide (8 μg in 4 μL 0.05% ascorbic acid in saline; Sigma) was infused at two sties along the medial forebrain bundle [site 1: AP -2.8 mm, ML -1.8 mm, DV -8.0 (all from Bregma); site 2: AP -4.7 mm (Bregma), ML -1.5 mm (midline), DV -7.9 mm (hole) according to (Paxinos and Watson, 1997)]. The infusion rate was 1 μL per min, and the cannula was left in place an additional 4 min to allow diffusion of the 6-OHDA solution. After surgery, the animals received subcutaneous saline, antibiotics (Duplocillin 0.1 ml/kg i.m.), and analgesia (Anafen 2 mg/kg s.e), and were kept warm in an incubator until fully recovered from anaesthesia. Animals were allowed to recover for at least 2 weeks after surgery before being treated with ECS.

Forelimb Use Asymmetry Test (Cylinder Test)

Animals were evaluated in the forelimb use asymmetry test (Cylinder Test; Schallert et al., 2000; Schallert and Woodlee, 2005) at three time points: before lesion, after lesion, and after ECS or sham treatment. At each time point, the animals were placed in a Plexiglas cylinder (20 cm diameter x 30 cm high) elevated on a glass plate for a 3 min period on two consecutive days. Testing was done during the dark phase of the cycle and under red lighting. The trials were videotaped from below, and scored at a later date by an investigator blind to the animals’ treatment. Forelimb placements on the walls of the cylinder were categorized as left independent, right independent, or simultaneous movements, and a forelimb use asymmetry score was calculated as:

Equation 5.1: [(ipsi + ½ both) divided by (ipsi + contra + both)] x 100

(Schallert and Woodlee, 2005)
where ipsi and contra refer to the forelimbs ipsilateral and contralateral to the 6-OHDA-induced lesion respectively. Animals had to make greater than 20 movements at any given time point for their data to be included in the analysis, and no animals were excluded based on this criterion.

**Tapered/Ledged Beam-Walking (TB) Test**

Animals were also evaluated before lesion, after lesion, and after ECS or sham treatment on a tapered/ledged beam-walking (TB) test, adapted with slight modifications from Schallert and colleagues (Schallert and Woodlee, 2005; Zhao et al., 2005a). The details of the test have been described in detail (Strome et al., 2006a). Briefly, the animals are trained to walk across a 165 cm long beam which progressively narrows as they approach the goal (their darkened home cage). Beneath the surface of the beam is a ledge, which allows the animals to make slips with their feet off the main surface of the beam without falling off, and therefore prevents postural compensation in lesioned animals. Taking a step with only 1 or 2 toes on the main surface of the beam (and the other 4 or 3 toes overhanging the ledge) is scored as a half footfault, while stepping with the entire foot on the ledge rather than on the main surface of the beam is scored as a full footfault. In between trials, the animal remains in its home cage with the lights in the room turned off for one minute for reinforcement. Normal animals make very few errors (footfaults), and those only occur on the narrowest section of the beam.

On the testing day, the animals were brought to the behavioural room at the start of the dark cycle (12:00 p.m.) and allowed to habituate for 15 - 30 min. Before testing, each animal is allowed one “refresher” trial, which is not videotaped. One TB test was made up of 5 consecutive trials, and each trial was videotaped from the rear to allow a clear observation of the hindlimbs, and scored at a later date by an investigator blind to the animal’s condition. For each hindlimb, the number of steps taken in each of the three sections (wide, medium and narrow portions of the beam) and the number of full and half footfaults in the 5 trials was determined and summed to obtain a single composite score per TB test. Because the scores represent mainly large or small proportions (# errors per steps), transforming the data via an arcsine transformation (Eq. 2) normalizes the data (Zar, 1999).

**Equation 5.2:** $p' = \frac{1}{2}[(\arcsin \sqrt{\frac{X}{n} + 1}) + (\arcsin \sqrt{\frac{X + 1}{n} + 1})]$

Where $X =$ # errors, and $n =$ # steps.

**Electroconvulsive Shock Treatment**

Animals were assigned randomly to the ECS or sham treatment groups, and were treated every day for 10 days between 08:00 and 11:00. Atropine sulfate (0.2 mg/kg s.c.) was administered, followed 30 min later by ketamine hydrochloride (80 mg/kg i.p.). After induction of ketamine anaesthesia, animals were given either sham treatment (electrodes placed, but no current administered), or bilateral ECS (80-99 mA, 5-9.9 s, 70 pulse/s, 0.5 ms pulse width) via earclip electrodes coated with electroconductive gel using a small animal ECS machine (Model 57800, Ugo Basile, Italy). All animals received the same initial current dose, based on our previous experience with ECS in rats under ketamine anaesthesia, and current doses during subsequent treatments were modified based on the nature of the previous seizure. All ECS-treated animals experienced seizures of 13-19 s duration. All animals in this study consistently showed tonic hind limb extension. One sham-treated animal from each of the D1 and D2/D3 binding groups was lost to ketamine anaesthesia.
Forty-eight hours after the last ECS, animals were decapitated and the brains were removed and quickly frozen in isopentane cooled with dry ice, and stored at -80°C until sectioning. Twenty micron coronal sections were cut at -18°C on a cryostat (Leica) and thaw-mounted onto glass microscope slides (Superfrost Plus, Fisher Scientific, Canada). The slides were stored at -80°C until the receptor binding assays were performed.

**Vesicular Monoamine Transporter-2 Binding**

For verification of the extent of lesion, coronal sections through the striatum were incubated with [11C](±)dihydrotetrabenazine, a marker for DA terminals, which binds to the vesicular monoamine transporter-2 (DaSilva et al., 1994). The details of the autoradiographic technique have been described in detail in Chapter 4.

**D1 Receptor Binding**

The slides were warmed up to room temperature, and pre-washed for 15 min in Tris-HCl buffer (50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, room temperature, pH 7.4, all from Sigma). Incubation was in 2 nM [3H]SCH 23390 (Perkin Elmer, Canada; specific activity 81 Ci/mmol) plus 30 nM ritanserin (to block 5-HT₂ receptors; Sigma), in the same buffer at 20°C for 45 min. Non-specific binding, was determined by incubating adjacent slices with an additional 10 μM (+)-butaclamol (Sigma). At the end of the incubation, the slides were washed for 2 x 3 min in fresh buffer at 4°C, dipped briefly in cold distilled water, and allowed to dry on the bench top overnight. After post-fixation in paraformaldehyde vapor under vacuum in a dessicator for 24 h (Liberatore et al., 1999), the slides were apposed to pre-erased tritium-sensitive phosphor screens (Fuji Medical Systems Inc., Stamford, CT) in standard film cassettes with [3H] microscales (Amersham, UK) for 3 days. On the third day, the screens were removed from the cassettes and scanned in the Cyclone phosphor imager at 600 dpi resolution.

**D2 Receptor Binding**

The slides were warmed up to room temperature, and pre-washed for 15 min in Tris-HCl buffer (50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, room temperature, pH 7.4, all from Sigma). The slides were incubated for 45 min in 3 nM [11C]raclopride (specific activity 959 or 1975 Ci/mmol at the start of incubation) in the same buffer at room temperature. Non-specific binding was determined by incubating adjacent slices with an additional 10 μM (+)-butaclamol. At the end of the incubation, the slides were washed for 3 x 1 min in fresh buffer at 4°C, dipped briefly in cold distilled water, and allowed to dry in a fume hood for 20 min. The slides were then apposed to Multisensitive storage phosphor screens (Perkin-Elmer) along with 11C standards prepared as previously described (Strome et al., 2005). The screens were scanned as above after 2 h of exposure.

**D3 Receptor Binding**

D₃ receptor binding was performed with R-(+)
7-Hydroxy-[3H]di-n-propyl-2-aminotetralin ([3H]7-OH-DPAT) as described by (Levesque et al., 1992) with minor modifications. The slides were warmed up to room temperature, and pre-washed for 3 x 5 min in a HEPES buffer (50 mM HEPES, 1 mM EDTA, 0.1% bovine serum albumen, 120 mM NaCl, all from Sigma). Incubation was in 1 nM [3H]7-OH-DPAT (Amersham, specific activity 115 Ci/mmol) in the same buffer at room temperature for 90 min. Non-specific binding, was
determined by incubating adjacent slices with an additional 10 μM (+)-butaclamol. At the end of
the incubation, the slides were washed for 3 x 1 min in fresh buffer at 4°C, dipped briefly in cold
distilled water, and allowed to dry on the bench top overnight. Post-fixation, exposure and plate
scanning were identical to that described for D1 binding, except exposure time was 7 d.

Data Analysis

Optical density analysis was performed using the inherent software on the phosphor
imager (Optiquant v4.00, Perkin-Elmer). DA receptor binding was measured in the nucleus
accumbens (NAcc) shell and in the dorsal striatum (approximately + 1.70 mm from Bregma
according to (Paxinos and Watson, 1997). Small regions of interest were placed bilaterally in at
least four total binding and two adjacent non-specific binding sections for each animal in each
region. The optical density data were converted to nCi/mg tissue using a standard curve derived
from the [3H] or [11C] microscales. For each animal, non-specific binding was subtracted from
total binding to get a measure of specific radiotracer binding.

Statistical Analysis

Repeated measures analysis of variance (ANOVA; treatment x time) was used for the
analysis of the behavioural data because multiple measurements were made in the same animals.
The effects of ECS treatment on DA receptor binding were evaluated using two-way (treatment x
hemisphere) ANOVA. Post-hoc testing of significant main effects was performed using Tukey’s
honest significant difference test for unequal n. All statistical analyses were performed using the
software program StatSoft Statistica '98 v5.1 (Tulsa, OK).

Results:

Cylinder Test

Figure 5.1 shows asymmetry scores from the Cylinder Test. There was no significant
effect of ECS treatment on forelimb use asymmetry.

Tapered/Ledged Beam-Walking Test

Figure 5.2 shows the results from the TB test. The animals mainly made mistakes in the
narrow section of the beam with their hindlimb contralateral to the lesion, so only these data are
shown. Repeated measures ANOVA indicates that there was a significant interaction effect
between treatment and time (F1.48 = 7.96, p < 0.007), and visual inspection of the data indicates
that the ECS-treated group had lower scores after treatment than the sham-treated group (Fig.
5.2).

Vesicular Monoamine Transporter-2 Binding

All animals showed > 90% depletion of vesicular monoamine transporter-2 binding in the
lesioned compared to intact dorsal striatum (mean ± SEM = 94.05 ± 1.89 %, data not shown, but
Fig. 4.4C, in Chapter 4, page 74 is representative).
Figure 5.1: Forelimb use asymmetry scores before and 48 h after repeated ECS or sham treatment. There was no significant effect of repeated ECS treatment. Values are mean ± SEM.

Figure 5.2: TB test data for the hindlimb contralateral to the lesion on the narrow section of the beam in ECS- and sham-treated rats before and 48 h after treatment. There is a significant treatment x time interaction (*p < 0.007), with ECS-treated rats showing lower scores after treatment, compared to sham-treated controls.
**D₁ Receptor Binding**

There was a significant effect of treatment on D₁ binding in the dorsal striatum (Two-way ANOVA: F₁,₃₄ = 5.20, p < 0.03; Figure 5.3a), with post-hoc testing indicating that D₁ receptor binding was significantly increased after ECS treatment (p < 0.04). There was also a significant effect of treatment on D₁ binding in the NAcc shell (F₁,₃₂ = 5.75, p < 0.03; Figure 5.3a), and again, post-hoc testing indicates that D₁ receptor binding was significantly increased after ECS treatment (p < 0.03). There were no significant main effects of hemisphere in these analyses, indicating that ECS treatment increased D₁ binding, regardless of the 6-OHDA lesion.

**D₂ Receptor Binding**

There were no significant main effects of treatment, indicating that ECS-treatment had no effect on D₂ receptor binding in either the dorsal striatum or NAcc shell. There was a significant effect of hemisphere on D₂ binding in the dorsal striatum (F₁,₃₄ = 47.45, p < 0.0001; Figure 5.3b), with post-hoc testing indicating that D₂ receptor binding was significantly increased in the lesioned hemisphere (p < 0.0002).

**D₃ Receptor Binding**

There was a significant effect of treatment on D₃ binding in the dorsal striatum (Two-way ANOVA: F₁,₃₄ = 4.55, p < 0.05; Figure 5.3c), with post-hoc testing indicating that D₃ receptor binding was significantly increased after ECS treatment (p < 0.05). There was also a significant effect of treatment on D₃ binding in the NAcc shell (F₁,₃₄ = 7.62, p < 0.01; Figure 5.3c), and again, post-hoc testing indicates that D₃ receptor binding was significantly increased after ECS treatment (p < 0.01). There was also a significant effect of hemisphere on D₃ binding in the NAcc shell (F₁,₃₄ = 4.55, p < 0.002; Figure 5.3c), with post-hoc testing indicating that D₃ receptor binding was significantly decreased in the lesioned hemisphere (p < 0.002).
D₁ Binding

A) 

\[ ^3H \text{SCH 23390 binding (nCi/mg tissue)} \]

<table>
<thead>
<tr>
<th>Condition</th>
<th>Dorsal Striatum</th>
<th>Ventral Striatum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lesioned</td>
<td>Bar</td>
<td>Bar</td>
</tr>
<tr>
<td>Non-lesioned</td>
<td>Bar</td>
<td>Bar</td>
</tr>
<tr>
<td>Lesioned</td>
<td>Bar</td>
<td>Bar</td>
</tr>
<tr>
<td>Non-lesioned</td>
<td>Bar</td>
<td>Bar</td>
</tr>
</tbody>
</table>

D₂ Binding

B) 

\[ ^11\text{C} \text{raclopride binding (nCi/mg tissue)} \]

<table>
<thead>
<tr>
<th>Condition</th>
<th>Dorsal Striatum</th>
<th>Ventral Striatum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lesioned</td>
<td>Bar</td>
<td>Bar</td>
</tr>
<tr>
<td>Non-lesioned</td>
<td>Bar</td>
<td>Bar</td>
</tr>
<tr>
<td>Lesioned</td>
<td>Bar</td>
<td>Bar</td>
</tr>
<tr>
<td>Non-lesioned</td>
<td>Bar</td>
<td>Bar</td>
</tr>
</tbody>
</table>
D_3 Binding

**Figure 5.3:** DA receptor binding values in the dorsal and ventral striatum in ECS- and sham-treated rats. A) D_1 binding was significantly increased after repeated ECS treatment in both the dorsal and ventral striatum (*p < 0.04). B) There was no effect of repeated ECS treatment on D_2 binding, but it was significantly increased in the lesioned dorsal striatum (#p < 0.001). C) D_3 receptor binding was significantly increased in both the dorsal and ventral striatum after repeated ECS treatment (*p < 0.04), and significantly decreased in the lesioned ventral striatum (#p < 0.002). N = 8-10 animals per group; values are mean ± SEM.
Discussion:

This study shows that repeated ECS treatment in unilaterally 6-OHDA-lesioned animals increases dorsal striatal and NAcc shell D1 and D3 receptor binding, without affecting D2 binding, and improves hindlimb motor performance on a beam-walking task.

The primary observation of the effects of ECT on PD patients is a fairly immediate and long-lasting improvement in their motor symptoms (Andersen et al., 1987; Zervas and Fink, 1991; Fall et al., 1995; Pridmore and Pollard, 1996). Very few studies, however, have examined motor behaviour after ECS treatment in rodents. Those reports looked at drug-induced behaviours (Wielosz, 1981; Green et al., 1983b; Smith and Sharp, 1997), with only one report in unilateral 6-OHDA-lesioned rats (Green et al., 1977), and all of these studies showed significant increases in DA-mediated behaviours after repeated ECS treatment. We hypothesized that non-pharmacological motor behaviour of unilateral 6-OHDA-lesioned rats would also improve after a course of ECS treatment. Our results indicate no significant effect of repeated ECS treatment on forelimb use asymmetry in the Cylinder Test (Fig. 5.1), but a significant improvement in hindlimb motor performance on the TB test (Fig. 5.2).

Considering the severity of the unilateral lesion (> 90% depletion of striatal DA terminals), the absence of an effect of ECS on the use of the forelimb contralateral to the lesion in the Cylinder Test was not surprising. After unilateral 6-OHDA lesioning, it is not necessary for the animals to use the impaired forelimb for many tasks, including exploration, and preferential use of the unimpaired forelimb has been shown to develop very rapidly and persist for a long period of time after severe unilateral 6-OHDA lesion (Evenden and Robbins, 1984; Dunnett et al., 1987; Miklyaeva et al., 1994). This preference for using the forelimb ipsilateral to the lesion makes it more difficult for the animal to “relearn” the use of the impaired forelimb, even when therapeutic interventions occur, especially in the case of mild or short-term therapeutic effects. Some recovery of impaired forelimb function in unilateral 6-OHDA-lesioned rats has been shown to occur after very specific interventions, such as DBS (Shi et al., 2004), lentivector delivery of GDNF to the striatum and SN (Dowd et al., 2005), or through forced use of the impaired forelimb (Tillerson et al., 2001), likely as a result of increased striatal GDNF (Cohen et al., 2003), interventions that either bypass the striatal DA deficit, and act to inhibit the overactive basal ganglia output structures to normalize motor cortex activity, or provide strong DAergic trophic support to the SN and striatum.

We did, however, see a significant improvement in TB test scores after repeated ECS treatment. The TB test however measures different aspects of locomotion and a lack of performance errors as opposed to an increase in use/performance as the cylinder test. Because the rat never has the choice to “ignore” its impaired hindlimb, it is likely that the degree of asymmetry between hindlimbs remains milder than for specialized movements of the forelimbs. Thus, a smaller contralateral improvement may be more likely to improve scores on this test. We have shown the relationship between performance on the task and the integrity of the striatal DA system (Chapter 4), and have used this task to measure improved motor performance after striatal cell transplantation in the unilateral 6-OHDA-lesioned rat (Cepeda et al., 2006). In addition, in a genetic mouse model of PD, L-DOPA treatment significantly improves performance on an adaptation of the test for mice (Hwang et al., 2005). The task is also widely used in models of stroke, where it has been shown to be sensitive to ischemic brain injury (Zhao et al., 2005a; Zhao et al., 2005b). In short, the TB test is a simple and valid test of gross motor function, and we have shown that repeated ECS treatment in unilateral 6-OHDA-lesioned rats improves performance on this task.
In this study, we have also shown that repeated ECS treatment in unilateral 6-OHDA-lesioned rats increases binding to specific DA receptor subtypes in both the dorsal striatum and NAcc shell. Binding to the D₁ and D₂ receptors was increased in both the lesioned and non-lesioned hemispheres after repeated ECS treatment (although the increase in D₁ binding was much smaller in the unlesioned versus lesioned striatum), whereas D₂ receptor binding was unchanged by ECS treatment. There were also specific effects of the 6-OHDA lesion on D₂ and D₃ receptor binding, with D₂ binding increased in the lesioned dorsal striatum, and D₃ binding decreased in the lesioned NAcc shell. D₂ receptor upregulation has been widely reported early after DA depletion in rodents (Graham et al., 1990; Narang and Wamsley, 1995), non-human primates (Doudet et al., 2000), and PD patients (Kaasinen et al., 2000), and the 6-OHDA-induced decrease in D₃ binding in the NAcc is also widely recognized (Levesque et al., 1995; Stanwood et al., 2000; van Kampen and Stoessl, 2003).

Our results on the effects of repeated ECS treatment on DA receptor binding in 6-OHDA-lesioned rats are consistent with the previous literature in normal rodents. Using both homogenate and autoradiographic receptor binding techniques, upregulation of D₁ receptors is a common finding in the normal striatum after a course of ECS (Fochtmann et al., 1989; Nowak and Zak, 1989; Barkai et al., 1990), while D₂ receptors are typically unchanged in the dorsal striatum (Bergstrom and Kellar, 1979; Reches et al., 1984; Martin et al., 1995), and D₂ and D₃ receptors have been reported to be upregulated in the NAcc (Barkai et al., 1990; Lammers et al., 2000). Our observations, then, of increased D₁ and D₃ binding, without concomitant changes in D₂ binding after repeated ECS treatment in 6-OHDA-lesioned rats are in good agreement with literature on the effects of repeated ECS on these receptors in normal animals.

The D₃ receptor is most abundant in the Islands of Calleja and NAcc, and is expressed at very low levels in the dorsal striatum and the rest of the rat brain under normal circumstances (Levesque et al., 1992; Diaz et al., 1995). Chronic treatment with L-DOPA in unilateral 6-OHDA-lesioned rats leads to behavioural sensitization (the rodent homolog of L-DOPA-induced dyskinesia) and a dramatic increase in the expression of the D₃ receptor in the lesioned dorsal striatum (Bordet et al., 1997; van Kampen and Stoessl, 2003). It appears that three conditions must be met to increase the dorsal striatal expression of the D₃ receptor in the rat brain after chronic pulsatile L-DOPA treatment: 1) severe 6-OHDA depletion of striatal DA, 2) activation of the D₁ receptor, and 3) elevated brain-derived neurotrophic factor (BDNF) levels (Bordet et al., 1997; Guillin et al., 2001; van Kampen and Stoessl, 2003).

Although the increased D₃ receptor binding that we observed in the dorsal striatum after repeated ECS treatment was less pronounced than in L-DOPA-induced behavioural sensitization (12-15% in this study, vs. 130-680%; Bordet et al., 1997; van Kampen and Stoessl, 2003), it may follow a similar mechanism. There is strong evidence that BDNF activity is increased after repeated ECS treatment, not only in the hippocampus (Nibuya et al., 1995; Altar et al., 2003; Jacobsen and Mork, 2004), but also in the striatum of both normal (Angelucci et al., 2002) and 6-OHDA-lesioned rats (Strome et al., 2006b). BDNF expression is regulated by cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB) (Shieh et al., 1998), and D₁ receptor activation leads to phosphorylation of CREB and the transcription of BDNF (Fang et al., 2003). CREB itself is upregulated in the hippocampus by repeated ECS treatment (Nibuya et al., 1996). Taken together, it seems reasonable to hypothesize that the ECS-induced increase in the D₁ receptor in the striatum could lead to increased CREB activity, leading to the transcription of BDNF, and increased expression of the D₃ receptor.

The fact that the D₃ receptor was only moderately upregulated by repeated ECS treatment compared to behavioural sensitization to L-DOPA treatment may, in fact, be advantageous. In
the rat, the induction of the D3 receptor in the dorsal striatum after chronic L-DOPA treatment occurs mainly in dynorphin/substance P (and D1) expressing neurons of the direct striatonigral pathway (Bordet et al., 2000). Co-expression of D1 and D3 receptors has been shown to have both opposite and synergistic effects on cAMP and on gene expression (Ridray et al., 1998). When the two receptor subtypes are in a synergistic relationship, the relative abundance of the receptors may dictate the functional outcome. For example, synergy between D1 and D3 occurs in L-DOPA-induced behavioural sensitization, but in this case, the D3 receptor is expressed at high levels, leading to overactivity of the direct pathway of the basal ganglia, and the development of sensitization (Bordet et al., 1997; Bordet et al., 2000). If D3 receptors are expressed in a low to moderate ratio compared to the D1 receptor, however, the synergy between them may enhance the activity in the direct pathway, without causing excessive stimulation. The nature of the synergistic relationship between D1 and D3 therefore may depend on the relative expression of the two receptor subtypes, with moderate levels of D3 being advantageous, and high levels detrimental. If ECT treatment enhances D3 expression only moderately, then activity in the direct pathway will be enhanced, but not excessive.

In conclusion, this is the first study to show improvements in non-pharmacological motor performance and increases in specific DA receptor subtypes after repeated ECS-treatment in 6-OHDA-lesioned rats. ECT is a non-invasive and safe treatment, and is widely used to treat psychiatric disorders. The clinical evidence suggests that in some patients, ECT can provide almost immediate and fairly long-lasting relief of the motor symptoms of PD. ECT should be considered in PD patients with poor response to medication, prior to surgical intervention in patients with severe motor symptoms, and, given its potential neurotrophic effects, perhaps even in patients early in the course of the disease. While the mechanism of action is not completely known, and further research is necessary, this study increases our understanding of the effects of ECT on the brain, and provides support for the continued use and study of ECT as a potential adjunct treatment for PD.
References:


Smith SE, Sharp T (1997) Evidence that the enhancement of dopamine function by repeated electroconvulsive shock requires concomitant activation of D1-like and D2-like dopamine receptors. Psychopharmacology 133: 77-84.


Chapter 6

THE EFFECTS OF ELECTROCONVULSIVE SHOCK ON GDNF, BDNF, AND FGF-2 CONCENTRATIONS IN THE 6-OHDA-LESIONED RAT BRAIN

Preamble:

In the previous chapter, we addressed two key aspects of our working hypothesis (Figure 1.1, page 10); the effects of repeated ECS treatment in unilateral 6-OHDA-lesioned rats on 1) motor function, and 2) DA receptors. In this chapter, we address another key feature of our working hypothesis, Hypothesis 3, that repeated ECS treatment in 6-OHDA-lesioned rats enhances striatal protein levels of the neurotrophic factors GDNF, BDNF, and FGF-2. We originally performed a small pilot study looking at the effects of repeated ECS on BDNF expression using in situ hybridization, and although we replicated the previously reported ECS-induced increase in piriform cortex BDNF mRNA, we were unable to detect BDNF mRNA within the striatum (see Appendix B for more detail). We therefore decided to move away from mRNA, and instead investigate neurotrophic factor protein levels directly in our regions of interest.

Introduction:

Parkinson’s disease (PD) is a progressive and debilitating neurodegenerative disorder affecting millions of adults worldwide. The hallmark neuropathological finding is the degeneration of midbrain dopamine (DA)-producing neurons, the loss of which leads to the characteristic motor symptoms of bradykinesia, tremor, rigidity and postural imbalance (Hornykiewicz, 1972). Various drug therapies exist, but these can cause debilitating side effects and/or lose their effectiveness after extended use (Jankovic, 2005). Surgical interventions, such as targeted lesions of the basal ganglia output structures that become overactive in PD (pallidotomy or subthalamotomy), or the implantation of stimulating electrodes to overexcite those same brain regions, and thereby inhibit their activity (deep brain stimulation), are becoming more common in advanced patients, but these are invasive treatments and carry inherent risks. As such, there is currently a need for the development of alternative treatments for PD.

One of the most effective and widely used treatments for depression, electroconvulsive therapy (ECT), appears to also improve the motor symptoms of PD patients, regardless of whether or not they are depressed. While the majority of reports on this phenomenon in the literature have been case reports, open trials, or retrospective studies, ECT treatment has consistently been shown to improve the main motor symptoms of PD, including rigidity and bradykinesia, and the improvements last from several weeks to months (Stern, 1991; Zervas and Fink, 1991; Fall et al., 1995; Pridmore and Pollard, 1996). To date, only one placebo-controlled double-blind trial on the effects of ECT in PD has been performed, but it showed that patients treated with ECT had prolonged “on” phases and decreased severity of their motor symptoms (Andersen et al., 1987).

ECT has mainly been investigated as an antidepressant treatment, and there is a large body of literature showing that it has very specific effects in cortical and hippocampal areas, limbic brain regions that are involved in mood disorders. The most consistent effects are: 1)
Increased activity in the serotonin and DA systems via changes in the concentration of these neurotransmitters and/or their receptors (Nowak and Zak, 1989; Zis et al., 1992; Yoshida et al., 1998a; Yoshida et al., 1998b; Burnet et al., 1999); 2) Increased neurotrophic factor levels (Nibuya et al., 1995; Angelucci et al., 2002; Gwinn et al., 2002); and 3) Enhanced neuronal growth (through either neuronal sprouting or neurogenesis; Gombos et al., 1999; Vaidya et al., 1999; Malberg et al., 2000; Scott et al., 2000; Lamont et al., 2001). We hypothesize that these effects of electroconvulsive shock (ECS – the animal model of ECT) are not limited to the limbic system, and that they may also occur in other brain regions, including areas involved in motor control. Although these effects may not lead to noticeable behavioural changes in subjects with an intact nigrostriatal system, they may account for some of the clinical improvements seen after ECT in parkinsonian patients. More specifically, in this study, we hypothesize that increased neurotrophic factor activity in the striatum may partially underlie the positive effects of ECS on the motor symptoms of PD.

To investigate this hypothesis, we studied unilaterally 6-hydroxydopamine (6-OHDA)-lesioned rats after repeated ECS or sham treatments. We then examined protein levels of glial cell line-derived neurotrophic factor (GDNF), brain-derived neurotrophic factor (BDNF), and basic fibroblast growth factor (FGF-2), three neurotrophic factors that are reduced in the brains of PD patients (Tooyama et al., 1993.; Mogi et al., 1999; Chauhan et al., 2001) and that are all protective against striatal DA loss in animal models of PD (Altar et al., 1994; Tomac et al., 1995; Shults et al., 1995; Bowenkamp et al., 1997; Shults et al., 2000). We chose to evaluate trophic factor levels in two limbic regions, the hippocampus and prefrontal cortex (PFC) as a control for the effectiveness of our ECS and immunohistochemical techniques, and in the striatum, the major target of the DA neurons that degenerate in PD, using the sensitive and specific immunohistochemical technique enzyme-linked immunosorbent assay (ELISA).

**Materials and Methods:**

**Subjects**

Adult male Sprague-Dawley rats [bred at the University of British Columbia animal facility from Charles River Canada (Montreal, PQ) stock], weighing 250 g at the start of the experiment were housed on a 12:12 light:dark schedule (with lights off at 12:00 p.m.), at constant temperature and humidity (21°C, 55%). Initial pilot studies were performed in 10 animals (n = 5 per group), and an additional 20 animals were added to the study later (n = 10 per group). Of the 30 animals, 3 were excluded due to poor lesions, 6 were excluded because they did not show consistent seizure activity, and 3 were lost to ketamine anaesthesia. Eighteen animals completed the study (n = 10 sham, 8 ECS). The animals had access to food and water ad libitum, and were housed in pairs. All procedures were approved by the University of British Columbia Committee on Animal Care.

**6-OHDA Lesioning**

After at least 5 days of habitation and handling, allowing them to reach a weight of at least 270 g, the rats received a right unilateral 6-OHDA-induced lesion of the DA nigrostriatal pathway. Desipramine hydrochloride (25 mg/kg i.p.; Sigma-Aldrich Canada, Oakville, ON) was administered 30-60 min before 6-OHDA infusion to protect noradrenergic terminals. Animals were anaesthetized with isoflurane in O₂ (4% for induction, 1% for maintenance), given atropine sulfate (0.05 mg/kg s.c.), and placed into a stereotaxic frame (Kopf). With the skull flat between lambda and Bregma, a 2% solution of 6-OHDA hydrobromide (8 μg in 4 μL 0.05% ascorbic acid in saline; Sigma) was infused at two sites along the medial forebrain bundle [site 1: AP -2.8 mm, ML -1.8 mm, DV -8.0 (all from Bregma); site 2: AP -4.7 mm (Bregma), ML -1.5 mm (midline), DV -7.9 mm (hole) according to (Paxinos and Watson, 1997)]. The infusion rate was
1 μL per min, and the cannula was left in place an additional 4 min to allow diffusion of the 6-
OHDA solution. After surgery, the animals received subcutaneous saline, antibiotics
(Duplocillin 0.1 ml/kg i.m.), and analgesia (Anafen 2 mg/kg s.c.), and were kept warm in an
incubator until fully recovered from anaesthesia. Animals were allowed to recover for at least 2
weeks after surgery before being treated with ECS.

**Cylinder Test**

Animals were evaluated in the forelimb use asymmetry test (Cylinder Test; Schallert et
al., 2000; Schallert and Woodlee, 2005) at three time points: before lesion, after lesion, and after
ECS or sham treatment. At each time point, the animals were placed in a Plexiglas cylinder (20
cm diameter x 30 cm high) elevated on a glass plate for a 3 min period on two consecutive days.
Testing was done during the dark phase of the cycle and under red lighting. The trials were
videotaped from below, and scored at a later date by an investigator blind to the animals’
treatment. Forelimb placements on the walls of the cylinder were categorized as left
independent forelimb use, right independent forelimb use, or simultaneous use of both forelimbs,
and a forelimb use asymmetry score was calculated as:

\[
\text{Equation 6.1: } \left( \frac{\text{ipsi} + \frac{1}{2}\text{both}}{\text{ipsi} + \text{contra} + \text{both}} \right) \times 100
\]

(Schallert and Woodlee, 2005)

where ipsi and contra refer to the forelimbs ipsilateral and contralateral to the 6-OHDA-induced
lesion respectively. Animals showing less than 70% asymmetry after lesion were excluded from
further analysis (2 animals in this study).

**Electroconvulsive Shock Treatment**

Animals were assigned randomly to the ECS or sham treatment groups, and were treated
every day for 10 days between 08:00 and 11:00 a.m. Atropine sulfate (0.2 mg/kg s.c.) was
administered, followed 30 min later by ketamine hydrochloride (80 mg/kg i.p.). After induction
of ketamine-induced anaesthesia, animals were given either sham treatment (electrodes placed,
but no current administered), or bilateral ECS (80-99 mA, 5-9.9 s, 70 pulse/s, 0.5 ms pulse
width) via earclip electrodes coated with electroconductive gel using a small animal ECS
machine (Model 57800, Ugo Basile, Italy). All animals received the same initial current dose,
based on our previous experience with rats receiving ECS under ketamine anaesthesia, and
current doses during subsequent treatments were modified based on the nature of the previous
seizure. Mean seizure length was 15 ± 1 s (mean ± SEM), and did not change significantly over
the course of ECS treatment (data not shown). Any animal that did not consistently show tonic
hind limb extension after ECS treatment was excluded from the analysis (6 animals).

**Tissue Processing**

Forty-eight hours after the last ECS treatment, and immediately following the last
behavioural session, animals were sacrificed by decapitation. Their brains were quickly
removed and dissected on ice. The hemispheres were divided and the left and right PFC
(anterior to approximately +3.0 mm from Bregma, but excluding the olfactory bulb and tract;
Paxinos and Watson, 1997), hippocampus and striatum were stored individually in cold, labelled,
pre-weighed microtubes. The tubes were immediately re-weighed, and stored at -80°C until
further processing with ELISA.

On the day of the ELISA, the microtubes were removed from the freezer and thawed on
ice. The tissues were diluted 100x w/v in ice-cold lysis buffer with freshly added protease
inhibitors [100 mM Tris-HCl, pH 7.2, 400 mM NaCl, 4 mM EDTA, 0.2 mM

103
phenylmethylsulfonyl fluoride, 0.2 mM benzethonium chloride, 0.05% sodium azide, 2% bovine serum albumen, 2 mM benzamidine, 40 U/ml aprotinin (Calbiochem, La Jolla, CA), 0.2% Triton X-100 (all Sigma unless otherwise noted), and homogenized on ice with ultrasonication (Sonic Dismembrator, 3x 5 s pulses @ 20 s intervals, power level 3; Fisher Scientific, Ottawa, ON). The tubes were then centrifuged at 10 000 g for 20 min at 4°C. The supernatants were removed, and total protein concentration in the supernatants was determined by a commercially available detergent-compatible kit (DC Protein Assay; Bio-Rad, Hercules, CA). Samples were further diluted 1:1 v/v in lysis buffer, kept on ice, and immediately used for ELISA.

**GDNF and BDNF ELISA**

All ELISAs were performed in 96-well microplates (Nunc-Immuno MaxiSorp Plates; Nalge-Nunc International, Rochester, NY). GDNF and BDNF concentrations were determined following the directions in the appropriate Emax ELISA kit from Promega (Madison, WI). Briefly, the microplates were coated overnight (at 4°C) with the appropriate capture antibody diluted in carbonate coating buffer (pH 8.2 GDNF, pH 9.7 BDNF). On the second day, the plates were blocked, and diluted tissue samples were added to the plate in triplicate, and incubated at room temperature with shaking (6 h GDNF, 2 h BDNF), allowing the antigen (neurotrophic factor) to bind to the capture antibody. Following washing, the plates had an Anti-Human polyclonal antibody added, were incubated (16 h at 4°C GDNF, 2 h at room temperature BDNF), washed again, and then incubated with shaking at room temperature with a secondary anti-Ig-Y antibody conjugated to horseradish peroxidase (2 h GDNF, 1 h BDNF). Finally, the substrate (hydrogen peroxide plus tetramethylbenzidine solution, room temperature) was added to produce the color reaction. After 15 (GDNF) or 10 (BDNF) min, the reaction was stopped with 1 N hydrochloric acid, and the plates were read immediately in a microplate reader at 450 nm (Bio-Tek Instruments Inc., Winooski, VT). Standard curves were created for each microplate (range 1000 – 15.6 pg/mL), based on pilot studies and the literature (Angelucci et al., 2002).

**FGF-2 ELISA**

For the determination of FGF-2 concentrations, an antibody pair from R&D Systems Inc. (Human FGF basic Duo-Set; Minneapolis, MN) was used, following the manufacturer’s instructions. Briefly, the microplates were coated with capture antibody diluted in PBS buffer, and incubated overnight at room temperature. The following day, plates were washed and blocked with PBS buffer containing 1% bovine serum albumen (Sigma). Following washing, the tissue samples were added in triplicate and incubated for 2 h at room temperature. The plates were washed again, the polyclonal detection antibody was added, and allowed to incubate for 2 h at room temperature, followed by plate washing. Streptavidin-horseradish peroxidase was added to the plate and incubated for 20 min. Following washing, the substrate solution (hydrogen peroxide plus tetramethylbenzidine solution, room temperature; R&D Systems Inc.) was added, and the color reaction was stopped after 20 min with 2 N sulphuric acid (R&D Systems Inc.). The plates were read immediately at 450 nm. A standard curve was included with each plate (4000-62.5 pg/mL).

**Data Analysis**

Cylinder Test data were analyzed with repeated measures analysis of variance (ANOVA; treatment x time) and the software program Statistica '98 (StatSoft, Tulsa, OK).

The best 2 out of 3 optical densities for each sample were used for further analysis (the point furthest from the mean was discarded). Some assays for some animals showed low reproducibility and the data were discarded, so although 18 animals completed the study, the N
values for each assay are 7-10 per group for the BDNF and FGF-2 ELISA. The GDNF ELISA was only performed on the pilot group of animals (n = 10) because the results were clear even with a small number of subjects.

Because many of the data points for the GDNF ELISA were below the standard curve, we were unable to fully quantify the data. Instead, because all the samples from a particular brain region were analyzed on the same microplate, the optical density values (corrected for total protein content), were compared, and the data are presented as mean ± SEM percent of control. Fully quantified data for BDNF and FGF-2 were corrected for total protein content, and are presented as mean ± SEM percent of control (sham-treated animals).

The effects of ECS on neurotrophic factor protein concentrations were evaluated using two-way ANOVA (treatment x hemisphere). Examination of all main effects was performed with post-hoc testing using Tukey’s test for unequal N.

Results:

**Cylinder Test**

The mean asymmetry score for the entire group before lesioning (n = 18) was 52.17 ± 1.58 % (mean ± SEM). All animals except for two (and these were excluded from the study) showed at least 70% asymmetry in forelimb use when tested 2 weeks post-lesion (mean ± SEM = 83.72 ± 2.15%; Fig. 6.1). Two-way ANOVA indicates no significant difference between the two treatment groups, but a significant effect of time (F232 = 124.01, p < 0.0001), with post-hoc analysis showing the asymmetry scores are significantly lower before lesioning (p < 0.001), but no significant difference before or after treatment (Fig. 6.1).

**GDNF ELISA**

There was a significant effect of treatment on GDNF in the PFC (F14 = 12.97, p < 0.003), with post-hoc testing showing ECS treatment significantly decreased the amount of GDNF protein in the PFC (p < 0.004; Fig. 6.2a). There was no effect of ECS in either the hippocampus or striatum (Fig. 6.2b, c). There was a significant effect of hemisphere in the striatum (F115 = 7.11, p < 0.02), with post-hoc testing indicating that the lesioned striatum had significantly increased GDNF in both the ECS and sham groups (p < 0.02; Fig. 6.2c).

**BDNF ELISA**

There was a significant effect of treatment on BDNF protein in all three brain regions examined (PFC: F131 = 4.94, p < 0.04, hippocampus: F130 = 6.61, p < 0.02; striatum: F132 = 5.86, p < 0.03), with post-hoc testing showing a significant decrease in the PFC (p < 0.03), and significant increases in the hippocampus (p < 0.02) and striatum (p < 0.03) (Fig. 6.3). There was also a significant effect of hemisphere in the PFC (F131 = 5.20, p < 0.03), with post-hoc testing showing the lesioned hemisphere having lower BDNF (p < 0.03; Fig. 6.3a).

**FGF-2 ELISA**

There was no effect of treatment on FGF-2 levels in the PFC, and a slight, but not significant (p < 0.09) effect in the hippocampus (Fig. 6.4). There was a significant effect of treatment on FGF-2 levels in the striatum (F130 = 12.99, p < 0.002; Fig. 6.4c). Post-hoc testing shows ECS treatment significantly increased FGF-2 protein in the striatum (p < 0.002).
Figure 6.1: Forelimb use asymmetry scores before lesioning, and before and 48 h after repeated ECS or sham treatment. All animals showed > 70% asymmetry after lesion, but there was no effect of treatment. (* pre-lesion scores are significantly lower than post-lesion or post-ECS, p < 0.001).
Figure 6.2: The effects of repeated ECS treatment on GDNF concentrations in the brain. ECS treatment has no effect in the hippocampus (b) or striatum (c), but significantly decreases GDNF protein in the PFC (a; * p < 0.05). There is a significant increase in GDNF in the lesioned striatum (c; # p < 0.05). Data are presented as mean ± SEM percent of control (sham treated, intact hemisphere).
Figure 6.3: The effects of repeated ECS treatment on BDNF concentrations in the brain. ECS treatment significantly increases BDNF protein in both the hippocampus (b), and the striatum (c), * p < 0.05. There is a significant decrease in BDNF in the PFC after ECS treatment (a; * p < 0.05), and a significant decrease of BDNF in the lesioned PFC (a; # p < 0.05). Data are presented as mean ± SEM percent of control (sham treated, intact hemisphere).
Figure 6.4: The effects of repeated ECS treatment on FGF-2 concentrations in the brain. ECS treatment has no significant effect on FGF-2 protein in the PFC (a), or hippocampus (b), but significantly increases FGF-2 in the striatum (c; * p < 0.05). Data are presented as mean ± SEM percent of control (sham treated, intact hemisphere).
Discussion:

This study shows that ECS treatment in unilaterally 6-OHDA-lesioned animals affects neurotrophic factor expression differentially, depending on the brain region, the protein under investigation, and the integrity of the DA system. That is, ECS treatment significantly decreased GDNF protein concentrations in the PFC, whereas BDNF concentrations were enhanced in the striatum and hippocampus, but decreased in the PFC, and FGF-2 concentrations were enhanced in the striatum. Independently of the effects of ECS, we also found significant effects of the lesion on neurotrophic factor levels, with increases in GDNF in the lesioned striatum, and decreases in BDNF in the lesioned PFC.

Although we were unable to directly measure the neurochemical effects of the 6-OHDA lesion, we did evaluate motor behaviour in the animals before and after lesion, and after ECS or sham treatment using the Cylinder Test. The Cylinder Test is widely being adopted as a reliable and valid non-pharmacological behavioural test in rodent models of PD (Tillerson et al., 2001; Shi et al., 2004; Iancu et al., 2005). Non-pharmacological behavioural testing in 6-OHDA-lesioned rats is preferable in studies looking at neurotrophic factors because DA-stimulating drugs such as apomorphine and amphetamine can induce GDNF, BDNF, and FGF-2 (Meredith et al., 2002; Guo et al., 2002; Mueller et al., 2006). Our results show that the animals had normal and symmetrical bilateral forelimb function before lesioning, and that forelimb use was significantly asymmetrical after the lesion. In other studies, the degree of forelimb use asymmetry in the Cylinder Test has been shown to correlate strongly with neurochemical measures of 6-OHDA-induced lesions, including striatal DA depletion and loss of tyrosine hydroxylase positive cells in the SN (Schallert and Tillerson, 2000; Iancu et al., 2005). The fact that the animals in our study showed such strong biases toward using the forelimb ipsilateral to the lesion suggests that they suffered extreme loss of striatal DA.

While the primary clinical observation after treating PD patients with ECT is an improvement in their motor symptoms, we saw no significant improvement in forelimb use asymmetry on the Cylinder Test after ECS treatment. The forelimb use asymmetries observed in our 6-OHDA lesioned rats in the Cylinder Test, however, were extreme, suggesting an almost complete loss of striatal DA. In such extreme lesions, recovery of function on this test may be difficult to accomplish without invasive therapy such as deep brain stimulation (Shi et al., 2004) or viral vector delivery of GDNF (Dowd et al., 2005). Thus, further investigation is needed into the use of behavioural tests in which lesion severity may not obscure small behavioural improvement. Indeed, using drug-induced rotational behaviour, Green and colleagues (1977) reported changes in motor behaviour in the unilateral 6-OHDA-lesioned rat model of PD after ECS treatment. The use of apomorphine and/or amphetamine in this model, however, can lead to changes in trophic factors independently of ECS (Meredith et al., 2002; Guo et al., 2002; Mueller et al., 2006), making the relationship between ECS and motor improvement more difficult to interpret. In a parallel study, we have, however, found improvements in motor function after repeated ECS using a ledged beam-walking test (Strome et al., 2006b), a test of spontaneous motor activity independent of pharmacological stimulation, practice, learning, and motivation (Schallert and Woodlee, 2005; Cepeda et al., 2006). This ledged beam-walking task appears to be more sensitive to discrete changes in motor function and may be a better test to uncover small changes in behaviour than the Cylinder Test.

We routinely administer ECS under ketamine anaesthesia in both our rodent (Strome et al., 2006a) and non-human primate studies (Strome et al., 2005). After evaluating the use of several anaesthetics for rodent ECS, we chose ketamine based on its ease of use, its applicability to the clinical situation (ECT is commonly administered under ketamine anaesthesia to patients allergic to barbiturates and to increase seizure duration; (Datto et al., 2002), and because, although, compared to data obtained in rodents receiving ECS without anaesthesia, ketamine
does attenuate the effects of ECS treatment on several of the main outcomes [e.g. hippocampal BDNF expression (Chen et al., 2001b) and cortical 5-HT2 receptor binding (our own, unpublished observations)], those effects are still significantly greater than in sham-treated animals. Indeed, ECT is always administered to patients under anaesthesia, so the use of anaesthesia for ECS in rodents, we believe, models the clinical situation more closely.

In this study, GDNF was significantly increased in the 6-OHDA-lesioned striatum, and BDNF was significantly decreased in the PFC ipsilateral to the 6-OHDA lesion. The effect of the lesion on striatal GDNF was small (13.05% and 11.95% increases in the sham and ECS groups, respectively) whereas the decrease that we observed in BDNF in the PFC of the lesioned hemisphere (-21.28% and -31.44% increases in the sham and ECS groups) was more substantial. The evidence for 6-OHDA-induced changes in GDNF, BDNF or FGF-2 protein is minimal, and the existing reports are conflicting. While our results are in good agreement with one study showing increased GDNF, but no changes in BDNF and FGF-2 in the lesioned striatum of young rats (Nakajima et al., 2001), they do not agree with another showing increased striatal BDNF and GDNF in young, but decreased striatal BDNF in old rats after 6-OHDA lesions (Yurek and Fletcher-Turner, 2001). Either of the lesion effects that we observed may be compensatory reactions to the unilateral 6-OHDA lesion, as the system attempts to maintain homeostasis in the face of severe DA depletion. For example, the substantial decrease in the PFC ipsilateral to the 6-OHDA lesion may have resulted from the fact that most striatal BDNF is anterogradely transported from the frontal cortex (Altar et al., 1997), and with a loss of negative feedback inhibition from striatal DA terminals, the release of BDNF from corticostriatal projections may have been upregulated.

In recent years, various techniques have identified changes in neurotrophic factors as one of the most consistent and robust effects of repeated ECS treatment. Gene microarray studies have shown that, while the expression of many genes is altered by ECS, some specific pathways are affected. In particular, in the hippocampus and cerebral cortex, growth-promoting genes appear to be enhanced by ECS, including genes for angiogenesis, neurogenesis, immediate-early genes, BDNF and its downstream signaling molecules, and FGF-2 (Newton et al., 2003; Altar et al., 2004). Indeed, one of the most widely reported effects of ECS is increased expression of BDNF (both mRNA and protein), particularly in the hippocampus, cortical regions, and the striatum (Nibuya et al., 1995; Zetterstrom et al., 1998; Angelucci et al., 2002; Altar et al., 2003; Jacobsen and Mork, 2004). Although BDNF has been the most thoroughly investigated neurotrophin with respect to the effects of ECS, mRNA and protein expression of FGF-2 have also been shown to increase in the cortex and hippocampus after minimal ECS (Follesa et al., 1994; Gwinn et al., 2002; Kondratyev et al., 2002). In contrast, while ECS treatment increases mRNA for the GDNF receptors GFRα-1 and GFRα-2 in the hippocampus and parietal cortex, mRNA for the protein itself appears to be unaffected by ECS treatment (Chen et al., 2001a). So far, only one study has looked at GDNF protein after ECS (Angelucci et al., 2002), and it showed significant decreases in both the striatum and hippocampus, but no changes in the frontal or occipital cortices.

Although there are some technical differences between our study and most of the literature (e.g. we used ketamine anaesthesia for ECS and sacrificed the animals 48 hrs after the last ECS or sham treatment, and all of the studies cited above did not use anaesthesia and sacrificed the animals 24 hrs after the last treatment, our data on the effects of repeated ECS treatment in 6-OHDA-lesioned animals are generally consistent with the literature on the effects of ECS on neurotrophic factor expression in the normal rodent brain. Two observations, however, the significant decrease of BDNF in the PFC, and the effects of repeated ECS on GDNF protein in the brain regions examined in this study, have not been reported previously, and deserve further attention.
Our finding that repeated ECS decreased BDNF in the PFC was unexpected since the literature shows increases (Altar et al., 2003; Jacobsen and Mork, 2004) or no change (Angelucci et al., 2002) in frontal cortex BDNF protein after ECS treatment in normal rats. Whereas most previous studies looked at a large area of the frontal cortex, however, we examined neurotrophic factor concentrations in a very discrete region, the most anterior 2-3 mm of the cortex. We chose this specific region because in the rat, it receives fairly extensive DA innervation from the midbrain (Berger et al., 1991) and because neurons in this region also make direct projections to the striatum (Berendse et al., 1992). The significant decrease in BDNF in the PFC that we found in this study may arise from the fact that BDNF can be transported anterogradely from the PFC to the striatum (Altar et al., 1997). BDNF is released in an activity-dependent manner (Kojima et al., 2001; Balkowiec and Katz, 2002; Goggi et al., 2003), so the stimulation of the cortex by ECS treatment may have caused BDNF stores to be depleted in the PFC and released into the striatum, and our observation of a reciprocal change in BDNF concentrations in the PFC and striatum after repeated ECS treatment.

The effects of ECS on GDNF concentrations in the brain have only been examined in one previous report in normal animals (Angelucci et al., 2002), and this study showed decreases in the hippocampus and striatum, and no changes in the frontal and occipital cortices. The fact that we saw a significant decrease in GDNF in the PFC after ECS treatment, while Angelucci and colleagues saw no change in the frontal cortex, may, again, be due to differences in the regions being investigated. The other discrepancies between these two reports may be explained by the nature of GDNF trafficking, and the fact that we examined GDNF levels 48 hrs after the last ECS treatment, while Angelucci and colleagues measured it at 24 hrs post-ECS. Studies with radioactive GDNF show that it redistributes quickly after intraventricular injection in the rat brain (within 1 hr) and that by 7 days post-injection, it accumulates in specific brain regions, including the substantia nigra (SN), the hypothalamus, the fimbria and the cerebellum (Lapchak et al., 1997). So while we saw decreases or no changes in our regions of interest, there may have been increases in GDNF in areas receiving input from these regions (such as the hypothalamus, cerebellum, fimbria, or SN). In this study, we were not able to measure neurotrophic factor concentrations in the SN because of the small size of this region. While we may have been able to dissect a whole midbrain region, in our animals one SN was lesioned while the other was intact, and it was not possible to clearly separate the two hemispheres at that level during our dissections. It will be very important in future studies to measure neurotrophic factor changes in the SN of parkinsonian animals after repeated ECS treatment.

In recent years, evidence of the beneficial effects of trophic factors on neuroprotection and survival of DA neurons in cell culture, as well as in animal models, has led to their investigation as potential treatments for PD. Several different delivery methods have been investigated, including gene delivery via viral vectors (Bjorklund et al., 2000; Wang et al., 2002; Eslamboli et al., 2005), and direct infusion of the protein into the target area [e.g. intraputaminal infusion of GDNF (Gill et al., 2003; Patel et al., 2005; Slevin et al., 2005; Lang et al., 2006)]. However, this line of investigation faces significant barriers because of the large size of the proteins, their inability to cross the blood-brain-barrier, and hence the necessary invasiveness of the treatment. Currently, safety concerns are prohibiting the use of viral vectors in human patients, and one Phase II GDNF direct infusion study was halted due to a lack of statistically significant improvement at the dose used and the appearance of neutralizing antibodies in several patients (Lang et al., 2006). While the therapeutic potential of neurotrophic factors in treating PD and other diseases remains of great interest, clearly much more work must be done before their use is a clinical reality.

In contrast, ECT treatment may be a non-invasive method of delivering endogenously produced neurotrophic factors to the brain regions that lose their normal trophic support in
parkinsonism. Taking into account the published literature on trophic factors, our data suggest that part of the mechanism of action of ECT in PD may be through the enhancement of striatal BDNF and FGF-2 concentrations by either limiting cell death or promoting cell growth. Future studies are required to investigate the true relationship between increased trophic expression and behavioural improvement. As mentioned earlier, the choice of behavioural tests to use in animals with severe 95% lesion may be crucial and needs to be carefully considered. Future studies should examine the neurotrophic effects of ECS in the parkinsonian brain in more detail to determine specifically if and how increased BDNF and/or FGF-2 may enhance motor and striatal DA terminal function, and what the effects of ECS are on neurotrophic factor concentrations in the SN. We hypothesize that increased neurotrophic activity may have direct effects on striatal DA neurotransmission. BDNF activity plays a direct role in the expression of D₃ receptors in the striatum (Guillin et al., 2001), and may also influence the expression of the D₁ receptor (Do and Kuzhikandathil, 2005). We have recently shown that repeated ECS in 6-ODA-lesioned rats enhances striatal D₁ and D₃ receptor binding (Strome et al., 2006b), but further studies are required to determine if this is a result of enhanced BDNF concentrations in the striatum.

In conclusion, this is the first study to investigate the effects of repeated ECS treatment on the concentrations of GDNF, BDNF, and FGF-2 in the brains of 6-OHDA-lesioned rats. We have shown that repeated ECS has region- and protein-specific effects, decreasing GDNF in PFC, increasing BDNF in the striatum and hippocampus, but decreasing BDNF in the PFC, and increasing FGF-2 in the striatum. ECT is widely available as a treatment for psychiatric disorders, and is non-invasive, and safe to use. The clinical evidence strongly suggests that in some patients, ECT can provide almost immediate and fairly long-lasting relief of the motor symptoms of PD. While the mechanism of action is not completely known, and further research is necessary, this study, showing that ECS enhances striatal neurotrophic factor levels, is the first step to the clinical realization of ECT as an effective adjunctive therapy for PD.
References:


Strome EM, Zis AP, Doudet DJ (2006) Electroconvulsive shock enhances D\textsubscript{1} and D\textsubscript{3} receptor binding and improves motor performance in 6-OHDA-lesioned rats. Submitted.


Chapter 7

General Discussion

Preamble:
The ultimate goal of this body of research was to investigate the observation that repeated ECT treatments in patients with PD has a dramatic effect on their motor symptoms, improvements that usually take only a few treatments to emerge, and that last for several weeks. Based on the known neurochemical deficits in PD and the anatomy of the main system involved, the basal ganglia, and taking into account the literature on the effects of repeated ECS treatment in the normal brain, and the proposed mechanism of action of ECT in depression, we developed a working hypothesis to explain how ECT treatment can improve the motor symptoms of PD. In order to address the various aspects of our working hypothesis, however, it was necessary to first identify the appropriate animal models and tools to use. We performed several pilot and validation studies (Chapters 2-4 and Appendices A and B) to identify and develop those animal models and techniques. Only with the right tools at our disposal could we move forward to undertake studies directly pertaining to our working hypothesis regarding the mechanism of action of ECT in PD (Chapters 5 and 6). Taken together, this body of work tells the story of a journey of discovery, focused on examining the effects of ECS on the brain, but encompassing different animal models, different neurochemical systems, and a variety of tools, ranging from non-human primates to rats, 5-HT to DA to neurotrophic factors, PET to behavioural analysis, autoradiography to immunohistochemistry. This final chapter will summarize the findings, explore outstanding issues, discuss the limitations and significance of the research, and look towards the future.

Model and Tool Development:

Model Development: Antidepressant Treatments and the 5-HT$_2$ Receptor

In developing our models and tools, we relied heavily upon the observation that the 5-HT$_2$ receptor undergoes specific plastic changes after particular antidepressant interventions. In rodents and humans, chronic treatment with many classes of antidepressant drugs, including tricyclic antidepressants (TCAs), monoamine oxidase inhibitors, some SSRIs, and atypical antidepressants, downregulation of 5-HT$_2$ receptors appears to be the predominant effect (Bergstrom and Kellar, 1979a; Tang et al., 1981; Peroutka and Snyder, 1990; Todd et al., 1995; Attar-Levy et al., 1999; Yatham et al., 1999; Mischoulon et al., 2002). In contrast, in rodents, increased 5-HT$_2$ receptor binding and mRNA expression are seen after chronic ECS (Bergstrom and Kellar, 1979b; Kellar and Stockmeier, 1986; Biegon and Israeli, 1987; Butler et al., 1993; Burnet et al., 1995). These specific and widely reproduced changes to the 5-HT$_2$ receptor gave us a context to work within as we examined the use of non-human primates to investigate ECS-induced changes in neurochemistry (Chapter 2), the use of anaesthesia for rodent ECS (Appendix A), and the use of PET tracers for in vitro autoradiography (Chapter 3).

In Chapter 2, we show that, like antidepressant drug treatment in humans and rodents, repeated ECS in non-human primates decreases cortical 5-HT$_2$ binding. This study was particularly significant because it was the first to examine the effects of electroconvulsive stimuli on 5-HT$_2$ receptors in an organism phylogenetically higher than a rodent. The upregulation of the 5-HT$_2$ receptor in the rodent brain after repeated ECS is somewhat of an anomaly in the antidepressant literature, and is counterintuitive. If antidepressant treatments act to enhance 5-HT, then the post-synaptic 5-HT$_2$ receptor should downregulate to compensate. We suggested that the reason ECS in rats was consistently reported to increase 5-HT$_2$ receptor binding, instead
of decreasing it as in primates, may have been partially due to the fact that ECT is administered under anaesthesia to humans and non-human primates, but typically without anaesthesia to rats. As part of our pilot study investigating the use of anaesthesia for rat ECS, we also investigated if anaesthesia does have an impact on ECS-induced changes in the 5-HT$_2$ receptor (Appendix A). That study confirmed that, even under anaesthesia, repeated electroconvulsive stimulation in rats increases cortical 5-HT$_2$ receptor binding. For our purposes, this was encouraging, in that we could confidently administer ECS to rats under ketamine anaesthesia and still elicit one of the hallmark effects. In screening the literature more thoroughly, several other reports emerged where ECS had been given to rodents under halothane anaesthesia, and these all also show 5-HT$_2$ upregulation (Goodwin et al., 1984; Metz and Heal, 1986; Burnet et al., 1995; Burnet et al., 1999). None of these reports, however, presented a satisfactory explanation for the unexpected upregulation of the 5-HT$_2$ receptor after ECS in rats.

There are several possible explanations for the differential regulation of 5-HT$_2$ receptors after repeated electroconvulsive stimulation in different species. First of all, the basal state of the system may strongly influence the effects exogenous treatments have on 5-HT neurotransmission. The state of cortical 5-HT$_2$ receptors in depressed or remitted patients appears to be somewhat context-dependent, showing increases, decreases, or no changes in specific patient populations. Post-mortem studies of suicide victims or PET studies of patients with severely pessimistic attitudes show increased prefrontal cortex 5-HT$_2$ binding (Yates et al., 1990; Hrdina and Vu, 1993; Meyer et al., 2003). Antidepressant drug naive patients (Meltzer et al., 1999), and those studied in their first episode (Meyer et al., 1999) do not show changes in cortical 5-HT$_2$ receptor binding, while those with chronic or recurrent depression show widespread decreases in cortical 5-HT$_2$ binding (Yatham et al., 2000; Larisch et al., 2001). We did not investigate the effects of ECS on 5-HT$_2$ receptors in an animal model of depression, nor, for that matter, did any of the previous investigators of ECS-induced changes in 5-HT$_2$ receptors in rodents. The normal state of the 5-HT system before ECS treatment in these animals could influence the plasticity of the system, and further investigations of the effects of antidepressants on 5-HT neurotransmission in animal models of depression are warranted.

Secondly, species differences in cortical neuroanatomy and, in particular connectivity, are not insignificant (reviewed in Kaas, 1987). As a treatment, ECT is highly dependent on cortical circuitry to exert its effects, and the differences between the primate and rodent cortex may contribute to the unexpected upregulation of 5-HT$_2$ receptors by ECS treatment.

Finally, that TCAs and ECT exert opposite effects on 5-HT$_2$ receptors in the rat brain may result from the fact that they have different mechanisms of action. Indeed, while the decreases in 5-HT$_2$ receptor binding induced by the TCAs in the frontal cortex as measured by $[^3]$H]ketanserin is in the order of 40% (Staton et al., 1986; Geretsegger et al., 1998; Strome et al., 2005), the effects of ECS are more subdued, 16% in our study (Fig. A.4) and typically 20% in the literature (Pandey et al., 1992; Butler et al., 1993). While ECT is thought to exert its effects through direct enhancement of synaptic 5-HT (Zis et al., 1992; Yoshida et al., 1998; Juckel et al., 1999), TCAs, along with their noradrenergic reuptake inhibition properties, may also be acting directly as 5-HT$_2$ antagonists (Sanchez and Hyttel, 1999). Antidepressant treatments may then be considered to act by modulating different aspects of 5-HT neurotransmission, and either influence 5-HT$_2$ receptor expression directly, as in the case of TCAs (direct 5-HT$_2$ antagonists), or indirectly, through ECS-induced changes in synaptic 5-HT (indirect 5-HT$_2$ agonists).

From a clinical standpoint, however, it may not be important what effects these various antidepressant treatments have on 5-HT$_2$ receptors. We use 5-HT$_2$ receptors in research as an indirect means to infer the effects of interventions on synaptic levels of their endogenous ligand, 5-HT. If the serotonin hypothesis of depression holds, then any treatment that increases the synaptic availability of 5-HT in target regions should have a beneficial effect. ECS or ECT may
to releasing 5-HT through widespread electrical stimulation of the brain, and TCAs may increase synaptic 5-HT indirectly, by inhibiting reuptake. If the net effect of diverse antidepressant treatments is the same, that is increased synaptic 5-HT, if it occurs via different mechanisms, then the use of 5-HT2 receptors as an indirect marker for synaptic levels of 5-HT may be misleading.

The fact that antidepressant treatments can have opposing effects on 5-HT2 receptors and still be effective treatments speaks to the incredible plasticity of monoamine neurotransmitter systems, and suggests that a variety of mechanisms have evolved to compensate for changes in neurochemical activity.

**Tool Development: Autoradiography, Behavioural Analysis, Measuring Neurotrophin Factors**

In our MPTP-treated non-human primates, the effects of repeated ECS treatment on motor behaviour were dramatic and fairly long-lasting (Doudet et al, unpublished). The effects on DA neurotransmission, however, were less clear, and were difficult to interpret in our small sample size. To pursue this line of investigation further, it was necessary to move on to a small animal rodent model, where larger numbers of subjects can be employed without the same ethical and financial limitations of non-human primate research.

While we moved to a rodent model, we still wanted to employ the tracers for the DA system used in vivo in PET. At the time, we did not have access to a small animal PET scanner, so instead, we decided to use the tracers for in vitro autoradiography. One of the major technical projects of this doctoral research, then, was the development and validation of a quantitative in vitro technique for using PET tracers autoradiographically (whereas most previous autoradiographic work with these tracers had been qualitative). In Chapter 3, we describe, in detail, the procedure for performing the binding assay with PET tracers, the quantification and analysis of the data, and our determination of the resolution of the technique. To validate the technique, we treated rats chronically with the antidepressant desipramine, which downregulates cortical 5-HT2 receptors (Bergstrom and Kellar, 1979a; Goodnough and Baker, 1994), to show that quantitative autoradiography with a PET tracer for 5-HT2 receptors gives data that are not significantly different than the traditional tritiated ligand for this receptor.

In the last several years, one of the major projects in our laboratory has been the development and validation of a battery of non-pharmacological behavioural tests for rat models of PD. The TB test is a new beam-walking task that is both designed to prevent the animals from making postural compensations and masking improvements in motor function, and is simple for the animals to learn (Schallert and Woodlee, 2005). The relationship between the integrity of the DA system and performance on the task, however, had not been examined previously. In Chapter 4, we show that, indeed, in unilaterally 6-OHDA-lesioned rats, TB test scores are correlated with striatal DA terminal integrity as measured by autoradiography.

Among neurotrophic factors, BDNF has two very unique characteristics: 1) its expression is activity-dependent (Kojima et al., 2001; Balkowiec and Katz, 2002; Goggi et al., 2003), and 2) it can be transported retrogradely from cortical areas to subcortical targets (Altar et al., 1997). Both of these characteristics make it a prime target for investigators examining the effects of ECS on the brain (see Table 1.3 in Chapter 1), because ECS causes widespread neuronal activity and because the primary stimulus is applied to the cerebral cortex. In Appendix B, we used in situ hybridization in an attempt to measure BDNF mRNA in the rat brain after ECS treatment. While no prior reports on ECS-induced changes in striatal BDNF mRNA existed, we wanted to investigate this phenomenon ourselves, since striatal transcription of the neurotrophic factors was a key aspect to our early working hypothesis. Our inability to detect BDNF mRNA in the striatum, while at the same time reproducing the widely reported increase in piriform cortex
BDNF mRNA expression after repeated ECS treatment, led us to modify our working hypothesis, and to examine neurotrophic factor protein levels directly in our later study.

Thus, in our early pilot and validation studies, we explored the use of non-human primates and PET imaging to examine the neurochemical effects of repeated ECS treatment, developed and validated a technique for in vitro quantitative autoradiography with PET tracers, investigated the use of anaesthesia for rat ECS, showed that the TB test is a DA-dependent motor task, and determined that in situ hybridization was not the best tool for investigating the effects of repeated ECS treatment on striatal levels of neurotrophic factors. These early studies allowed us to determine the most appropriate animal models and tools to use to address our specific hypotheses regarding the mechanism of action of ECT in PD.

The Effects of Repeated ECS on the DA System in Parkinsonian Rats: Status of Working Hypothesis

The major goal of this body of work was to perform several key studies examining the mechanism of action of ECT in PD. In particular, our working hypothesis identified three specific areas of investigation: motor behaviour, DA receptors, and neurotrophic factors. Our experiments addressing those hypotheses have shown that repeated ECS treatment in unilateral 6-OHDA-lesioned rats: 1) improves hindlimb, but not forelimb, motor function (Chapter 5); 2) increases D1 and D3 receptor binding in the dorsal and ventral striatum, without affecting D2 receptor binding (Chapter 5); and 3) enhances BDNF and FGF-2, but decreases GDNF protein levels in the striatum (Chapter 6).

**Hypothesis 1): Repeated ECS improves motor performance in 6-OHDA-lesioned rats**

In Chapter 5, we showed that, while repeated ECS treatment in unilateral 6-OHDA-lesioned rats did not improve forelimb function in the Cylinder Test, hindlimb performance on the TB test was significantly improved compared to sham-treated controls.

In hindsight, the lack of improvement on the Cylinder Test was not surprising, considering the extent of the lesion in this rat model of PD. In the unilateral 6-OHDA lesion model of PD, the loss of DA innervation to the ipsilateral striatum is extreme, and all animals in our studies showed greater than 90% depletion of striatal DA terminals. The Cylinder Test is widely used to evaluate forelimb motor function in the unilateral 6-OHDA-lesion model of PD, and while all reports agree that the lesion itself causes a severe impairment in the forelimb contralateral to the lesion on this task, recovery of that function only occurs after very specific interventions, such as lentivector delivery of GDNF to the striatum and SN (Dowd et al., 2005), through forced use of the impaired forelimb (Tillerson et al., 2001), likely as a result of increased striatal GDNF (Cohen et al., 2003), or DBS (Shi et al., 2004). In contrast, striatal transplantation of fetal DA neurons, which provides a focal replacement of striatal DA does not improve forelimb function in the Cylinder test, even though drug-induced behaviours are enhanced (Bjorklund et al., 1994; Dowd and Dunnett, 2004). It appears, then, that this test is only sensitive to antiparkinsonian interventions that either a) provide strong DAergic trophic support to the SN and striatum (and presumably restore some of the lost DA innervation), or b) bypass the striatal DA deficit, and act to inhibit the overactive basal ganglia output structures to normalize motor cortex activity. Synthesizing the literature, our inability to detect an improvement on the Cylinder Test may suggest that repeated ECS treatment in unilateral 6-OHDA-lesioned rats does not induce a major reinnervation of the lesioned striatum.

We did, however, detect a significant improvement in test scores on the TB test in ECS-treated rats compared to sham-treated controls (Chapter 5). While the TB test measures the number of hindlimb footfaults off the main surface of the beam, this task evaluates more than just the placement of the limb, requiring a whole host of skills to perform, including balance,
coordination, and motivation. In that sense, it is more of a measure of gross motor function than of either hindlimb or fine motor function. Given both the extremity of unilateral striatal DA depletion in our studies, and the non-invasive and indirect nature of ECS treatment, without using pharmacological agents to elicit DA-dependent motor behaviours, gross improvements in motor function are a noteworthy finding.

**Hypothesis 2): Repeated ECS upregulates striatal D₁ and D₃ receptors**

We show in Chapter 5 that repeated ECS treatment in unilateral 6-OHDA-lesioned rats has specific effects on the expression of DA receptors, upregulating D₁ and D₃ receptor binding in the dorsal striatum and NAcc shell, but having no effect on the D₂ receptor. Interestingly, the effects of repeated ECS were not different in the lesioned versus unlesioned striatum, which may result from the fact that the ECS treatment was applied bilaterally. We also observed specific changes in DA receptors as a result of 6-OHDA lesioning, with the D₂ receptor upregulated in the ipsilateral striatum, and the opposite effect on D₃ receptor binding in the ipsilateral NAcc shell, both changes that are widely reported in the literature after DA depletion (Levesque et al., 1992; Narang and Wamsley, 1995; Doudet, 2001; van Kampen and Stoessl, 2003).

Based on the previous literature of the effects of repeated ECS on DA receptors in the normal rat brain, we expected to find increased D₁ (Nowak and Zak, 1989; Sershen et al., 1991) and D₃ (Lammers et al., 2000) receptor binding in the dorsal striatum and NAcc shell, respectively, but this is the first report of increased D₃ binding in the dorsal striatum after repeated ECS treatment. Behavioural studies show that enhanced locomotor activity after repeated ECS treatment is only elicited by either mixed agonists (such as apomorphine; Green et al., 1977; Green et al., 1983), or co-administration of D₁-like and D₂-like agonists (Smith and Sharp, 1997). Since D₂ receptor binding is unchanged after repeated ECS (Bergstrom and Kellar, 1979b; Reches et al., 1984; Martin et al., 1995; Chapter 5), the motor activating effects of DA agonists may result from increased striatal D₁ and D₃ receptor binding.

The D₃ receptor is most abundant in the Islands of Calleja and NAcc, and is expressed at very low levels in the dorsal striatum and the rest of the rat brain under normal circumstances (Levesque et al., 1992; Diaz et al., 1995). Chronic treatment with L-DOPA in unilateral 6-OHDA-lesioned rats leads to behavioural sensitization and a dramatic increase in the expression of the D₃ receptor in the lesioned dorsal striatum, via activation of the D₁ receptor and increased BDNF levels (Bordet et al., 1997; Guillin et al., 2001; van Kampen and Stoessl, 2003). The increase in D₃ receptor binding that we observed in the dorsal striatum after repeated ECS treatment was less pronounced than in L-DOPA-induced behavioural sensitization, and yet it may follow a similar mechanism, since we have also shown increased D₁ receptor binding (Chapter 5) and elevated striatal BDNF (Chapter 6). Although we have not explicitly shown co-expression of the D₁ and D₃ receptors in this study, co-localization of these two receptor subtypes appears to be the norm whenever the D₃ receptor is expressed, for example in the dorsal striatum after chronic L-DOPA treatment (Bordet et al., 2000), and in the Islands of Calleja and NAcc shell under normal circumstances (Ridray et al., 1998).

One important question that arises from our working hypothesis is that, since D₁ and D₃ receptors have opposite effects on cAMP accumulation, how can their co-expression and co-activation enhance motor output? In fact, it appears that in some cases, the D₃ receptor can be coupled to another intracellular signal transduction pathway, that of mitogen-activated protein (MAP) kinase (Leigh et al., 1983; Cussac et al., 1999; Oldenhof et al., 2001). In addition, co-expression of D₁ and D₃ receptors has been shown to have both opposite and synergistic effects (Ridray et al., 1998). Ridray and colleagues (1998) have hypothesized that when the D₁ and D₃ receptors are acting synergistically, the D₃ receptor is actually coupled to MAP kinase.
We suggest that when the two receptor subtypes are in a synergistic relationship, the relative abundance of the receptors may dictate the functional outcome. For example, synergy between \( \text{D}_1 \) and \( \text{D}_3 \) occurs in L-DOPA-induced behavioural sensitization, but in this case, the \( \text{D}_3 \) receptor is expressed at high levels, leading to overactivity of the direct pathway of the basal ganglia, and the development of sensitization (Bordet et al., 1997; Bordet et al., 2000). If \( \text{D}_3 \) receptors are expressed in a low to moderate ratio compared to the \( \text{D}_1 \) receptor, however, the synergy between them may enhance the activity in the direct pathway, without causing excessive stimulation. The nature of the synergistic relationship between \( \text{D}_1 \) and \( \text{D}_3 \) therefore may depend on the relative expression of the two receptor subtypes, with moderate levels of \( \text{D}_3 \) being advantageous, and high levels detrimental. If ECT treatment enhances \( \text{D}_3 \) expression only moderately, then activity in the direct pathway will be enhanced, but not excessive.

**Hypothesis 3): Repeated ECS induces the trophic factors BDNF, GDNF and/or FGF-2 in the striatum of 6-OHDA-lesioned rats**

In Chapter 6, we show that the protein levels of the neurotrophic factors GDNF, BDNF, and FGF-2 are modified by repeated ECS treatment in 6-OHDA-lesioned rats. We originally hypothesized that all three factors would be increased in the brain regions we investigated (PFC, hippocampus, and striatum), but found that the effects were not as simple as that, and instead observed protein- and region-specific effects.

In unilateral 6-OHDA-lesioned rats, repeated ECS treatment increased BDNF protein in the hippocampus and striatum, and increased FGF-2 protein in the striatum. BDNF protein was significantly decreased in the PFC, and there were slight, but insignificant increases in FGF-2 in the hippocampus and PFC. GDNF was significantly decreased in the PFC, but was not changed elsewhere. We also detected specific effects of the 6-OHDA lesion on brain neurotrophic factor levels, with small increases in GDNF and FGF-2 in the striatum, and a decrease in BDNF in the lesioned PFC. In general, our findings were consistent with the literature on the effects of both 6-OHDA lesions and repeated ECS treatment in the normal rat brain, but there were a few exceptions.

BDNF protein was decreased in the PFC, a finding that we suggest might be explained by striatal release of BDNF from prefrontal corticostriatal projections. The majority of striatal BDNF protein arrives from the frontal cortex (Altar et al., 1997), and BDNF is released in an activity-dependent manner (Balkowiec and Katz, 2002). Since electroconvulsive stimuli primarily activate cortical neurons, repeated ECS treatment could result in an accumulation of BDNF in the striatum, and depletion of frontal cortex BDNF stores, as observed in our study. This same pathway may also underlie the lesion-induced decrease that we detected in BDNF protein in the PFC. As a compensatory response to the death of striatal DA terminals, the main striatal BDNF afferent pathway from the frontal cortex may have increased its activity.

We also found decreased GDNF protein levels in the PFC, but no changes in the hippocampus or striatum. GDNF is the most potent trophic factor for DA neurons, so our original hypothesis suggested that for GDNF to play a role in the mechanism of action of ECT in PD, its levels should be increased in the striatum after ECS treatment. What may be more important, however, is the concentration of GDNF in the SN. Studies with radioactive GDNF show that it redistributes quickly after intraventricular injection in the rat brain (within 1 hr; (Lapchak et al., 1997), and that by 7 days post-injection, it accumulates in specific brain regions, including the SN, the hypothalamus, the fimbria and the cerebellum (Lapchak et al., 1997). We examined GDNF levels 48 hrs after the last ECS treatment, which may have been enough time for the protein to redistribute. So while we saw decreased GDNF in the PFC, and no changes in the hippocampus, and striatum, there may have been increases in GDNF in areas receiving input from these regions (such as the hypothalamus, cerebellum, fimbria, or SN).
Synthesis of the Findings

Our results have generally provided support for our working hypothesis (updated here as Figure 7.1, which summarizes the results of these studies). An improvement in motor function is the primary observation after ECT treatment in patients with PD, and we have shown that 1) motor function is also improved in parkinsonian rodents after repeated ECS treatment. The basis for this improvement in motor function is presumably an enhancement of DA neurotransmission within the striatum, which may result from 2) increased binding to striatal D1 and D3 receptors.

The neurotrophic factors BDNF and FGF-2 can both enhance the survival and growth of DA neurons, and we have shown that 3) striatal BDNF and FGF-2 levels are enhanced in the striatum after repeated ECS treatment in 6-OHDA-lesioned rats. Individually, either of our observations on the effects of repeated ECS on striatal DA receptors or neurotrophic factor protein levels could explain improved motor function in parkinsonian animals, but together, they may interact to enhance DA function even further. A schematic of this hypothetical synergism between neurotrophic factors and the DA system is shown in Figure 7.2. Repeated ECS treatment increases basal striatal DA release (Zis et al., 1991), and if this DA release activates D1 receptors and the direct output pathway of the basal ganglia, then motor output is facilitated (Gerfen and Young, 1988; Albin et al., 1989; Gerfen et al., 1990). Repeated ECS treatment also likely causes increased striatal BDNF release. BDNF activity plays a direct role in the expression of the D3 receptor (Guillin et al., 2001), which is typically colocalized with the D1 receptor (Ridray et al., 1998). Activating the D3 receptor, then, also, leads to enhanced motor output. BDNF also enhances the survival of DA neurons, which could lead to even further increases in basal striatal DA release. Individually, ECS-induced striatal DA or BDNF release can facilitate motor output, but together, their combined effects may provide an even longer-lasting effect. This synergism may partially explain the fact that in PD patients, the effects of a course of ECT treatment on their motor function lasts for weeks to months (Friedman and Gordon, 1992; Fall et al., 1995; Pridmore and Pollard, 1996), and does not simply extinguish within hours or days after the end of treatment.

Limitations of the Studies

One of the primary limitations of this research is the use of the unilateral 6-OHDA-lesion rat model of PD. While this is the most commonly employed animal model of PD, some of the features are not representative of the clinical situation. In this model, the DA and motor deficits are induced in only one hemisphere of the brain and on one side of the body, and the unilateral loss of striatal DA terminals is extreme (> 90% depletion). In these ways, the model is a poor replication of the clinical reality, where patients suffer DA depletion and motor symptoms on both sides of their brain and body, and the disease is usually diagnosed around the time patients have lost approximately half of their nigrostriatal DA neurons and 80% of striatal DA (Bernheimer et al., 1973). A partial lesion model, using, for example, intrastrateal administration of 6-OHDA (Kirik et al., 1998), may have been a more appropriate choice for these studies. A major limitation of the neurotrophic factor study (Chapter 6) was the fact that we were unable to investigate changes in neurotrophic factor protein concentrations in the SN. While we may have been able to dissect a whole midbrain region, in our animals, one SN was lesioned, while the other was intact, and it was not possible to clearly and reproducibly separate the two hemispheres at that level during our dissections. Since all three of the neurotrophic factors that we investigated in this study are transported retrogradely from the striatum to SN (Mufson et al., 1999), where they help promote the survival of DA neurons, it will be very important in future studies to measure neurotrophic factor changes in the SN of parkinsonian animals after repeated ECS treatment.
Adapted from Duman et al., 1997.
Figure 7.1: Summary of the results of this body of work in the context of the working hypothesis. We have shown that repeated ECS treatment in unilateral 6-OHDA-lesioned rats: (1) improves the performance of the lesioned hindlimb on the TB test; (2) increases binding to striatal D₁ and D₃ receptors; and (3) enhances striatal protein levels of BDNF and FGF-2.
Repeated ECS treatment elevates basal striatal DA release, which could act via D₁ receptors to facilitate motor output. Repeated ECS treatment also increases striatal BDNF protein levels, which
In our examinations of repeated ECS treatment in unilateral 6-OHDA-lesioned rats, we chose to examine the behavioural and neurochemical effects at the specific time point of 48 hours after the last ECS treatment. We chose this time point based on the literature and on our own unique situation. Almost all of previous studies in the literature examined the effects of repeated ECS treatment on the rodent brain at 24 hours after the last ECS treatment, but the majority of these studies also did not administer ECS under anaesthesia. We wanted to ensure that any effects of anaesthesia had been eliminated before we tested the animals on complex behavioural tasks and, just as importantly, measured DA receptor binding, which has been shown to be affected by anaesthesia (Momosaki et al., 2004), and so we chose the time point of 48 hours to ensure that the anaesthetic had washed out. Examining the animals’ behaviour and neurochemistry at the 48 hour post-ECS time point, however, gave us only a very confined view of the effects of repeated ECS treatment. In contrast to our primate studies (Chapter 2), where we examined the effects of ECS longitudinally, giving the animals PET scans both early in the course of repeated ECS treatment and 24 hours, 1 week and 1 month after the end of treatment, we only investigated the behavioural and neurochemical effects of repeated ECS treatment in 6-OHDA-lesioned rats at a single time point. This limitation of our investigations means that we have gained no understanding of either the development or the duration of the effects of repeated ECS treatment in unilateral 6-OHDA-lesioned rats. In fact, there are very few publications examining the effects of ECS in animal models longitudinally. In rats, our own unpublished observations indicate that, in normal animals, improved performance on the TB test lasts up to 3 weeks after the end of treatment (Strome et al., unpublished). In agreement, Smith and Sharp (1997) found that the motor activating effects of simultaneous administration of D1-like and D2-like agonists lasts at least 3 weeks after the end of repeated ECS treatment. To provide further support for the use of ECT as an adjunct treatment for PD, it will be important to examine both the development and the duration of ECS-induced changes in parkinsonian animals in the future.

Finally, it is important to note that the evidence that we provide here for ECS-induced changes in parkinsonian animals is purely correlative. That is, we show that ECS causes specific effects in parkinsonian animals, but not that these effects are either necessary or sufficient to improve motor performance. A causal link between the improvement in motor performance observed in parkinsonian animals after repeated ECS treatment and changes in striatal DA receptors or neurotrophic factor levels can only be demonstrated by removing the receptor or neurotrophic factor. Various methods are commonly in use to perform such studies, including genetically modified mice [that either do not express (knockout) or have low expression (heterozygote) of a gene of interest], the use of antisense oligodeoxynucleotides against a specific gene of interest, which binds to the mRNA of that gene and prevents its translation, or the use of pharmacological antagonists, or antibodies against specific proteins. How some of these technologies might be applied to our research question is outlined below in “Directions for Future Study”.

Directions for Future Study

These studies are some of the first to look at the effects of ECS in an animal model of PD, and many questions remain unanswered regarding the mechanism of action of ECT in PD. Specifically, five important questions should be addressed in the future: 1) Do the D1 and D3 receptors underlie the improvement in motor function seen after ECS?, 2) What is the relationship between the increased D3 receptor binding and striatal BDNF activity?, 3) What is the mechanism for the increased striatal BDNF and FGF-2 concentrations?, 4) What is the role of CREB in ECS-induced changes in 6-OHDA-lesioned rats?, and 5) Does ECS treatment in 6-OHDA-lesioned rats promote survival or growth of striatal DA terminals?
To investigate the roles that the D₁ and D₃ receptors play, antisense oligodeoxynucleotides could be used to knock down the expression of these genes. This approach was taken by van Kampen and Stoessl (2000, 2003) to show that the behavioural sensitization that develops after chronic treatment with L-DOPA depends on D₁ and D₃ receptors. In our research model, using antisense to block D₁ and/or D₃ receptors, and then examining the effects of that knock down on motor improvement after ECS treatment would provide strong evidence of the role that these receptor subtypes play in mediating the positive effects of ECT on the motor symptoms of PD.

The relationship between the upregulation of DA receptors and increased BDNF activity after ECS could be addressed in the future using an antibody against the BDNF receptor, TrkB. This approach was used by Guillain and colleagues (2001) to show that the induction of the D₃ receptor in the dorsal striatum after behavioural sensitization to L-DOPA depended on BDNF signalling via TrkB. In our studies, we could infuse IgG-TrkB directly into the striatum during the course of repeated ECS treatment, and then examine the expression of D₁ and D₃ receptor mRNA and protein, as well as behaviour.

We have suggested that increased striatal neurotrophic factor concentrations after ECS treatment could arise from 2 sources; 1) anterograde release from afferent regions (such as the frontal cortex), or 2) the activation of striatal gene transcription and local release of trophic factors. At least for BDNF, we have shown that the latter alternative seems unlikely, since we were unable to detect BDNF mRNA in the striatum after repeated ECS treatment (Appendix B). One way to investigate the first theory is to perform aspiration lesions of the frontal cortex as a means of destroying the corticostriatal BDNF or FGF-2 projections. This approach has been used in two of the seminal studies of the relationship between DA neurotransmission, BDNF, and the D₃ receptor, to show that BDNF is transported anterogradely from the frontal cortex to the striatum (Altar et al., 1997), and that BDNF from the frontal cortex is necessary for L-DOPA-induction of the D₃ receptor in the dorsal striatum (Guillin et al., 2001). By destroying this pathway, and then examining the effects of repeated ECS on BDNF and FGF-2 protein levels in the striatum, the source of the proteins could be determined to be (or not to be) the frontal cortex.

An important component to our working hypothesis, but one that we have not addressed in these studies, is CREB. CREB is a transcription factor for many genes, including the neurotrophic factors, and it has been shown to play a crucial role in mediating the effects of chronic antidepressant and ECS treatment in the hippocampus (Nibuya et al., 1996; Thome et al., 2000; Chen et al., 2001). We suggest that CREB may also be important in the ECS-induced changes in the striatum. To investigate the role of CREB in the effects of repeated ECS in the parkinsonian brain, it is important to first examine whether or not CREB phosphorylation is increased in the striatum after repeated ECS treatment, which could be done using a specific antibody against phospho-CREB and immunoblotting (Newton et al., 2002). Duman’s group has used a tetracycline-regulated system to reversibly and selectively express the gene for CREB in specific brain regions of transgenic mice (Chen et al., 1998; Sakai et al., 2002), and this approach could also be used to investigate the role of CREB in the ECS-induced changes in the striatum of parkinsonian animals.

One of the most robust effects of ECS in the hippocampus is cell growth, in the form of both axonal sprouting (Gombos et al., 1999; Vaidya et al., 1999; Madhav et al., 2000; Lamont et al., 2001) and neurogenesis (Madsen et al., 2000; Malberg et al., 2000; Scott et al., 2000). In fact, while hippocampal neurogenesis can be stimulated by many antidepressant treatments (Malberg et al., 2000), mossy fibre sprouting appears to be unique to ECS (Lamont et al., 2001). Striatal DA terminals undergo sprouting in response to very specific stimuli, including partial or severe 6-OHDA or MPTP lesions (Blanchard et al., 1996; Bezard et al., 2000), D₂ receptor
antagonism or knockout (Parish et al., 2002), or infusion of GDNF and/or BDNF (Hudson et al., 1995; Lucidi-Phillipi et al., 1995; Batchelor et al., 2000). There is evidence for low levels of cellular proliferation in the striatum and SN under very specific circumstances, including D3 stimulation (van Kampen et al., 2004; van Kampen and Robertson, 2005). Considering that PD is characterized by a loss of a very specific subset of cells, patients suffering from this disease could greatly benefit from therapies that enhance cell survival or growth. As such, it will very important to address cell proliferation and axonal sprouting after ECS treatment in 6-OHDA-lesioned rats in future studies.

Significance of the Findings to PD

The most overt effect of ECS in PD patients is a dramatic improvement of their motor symptoms, so it was important to demonstrate improved motor function in our animal model. Moreover, DA receptors are important pharmacological targets in PD, with the standard drug treatments, L-DOPA or DA agonists, acting at these sites. Most PD patients who undergo ECT treatment must have their drug doses reduced or eliminated in order to avoid the development of negative side effects, such as dyskinesia (Zervas and Fink, 1992; Nymeyer and Grossberg, 2002), a phenomenon that could be explained by our observation of upregulated D1 and D3 receptors after repeated ECS in 6-OHDA-lesioned rats. Indeed, the fact that DA receptors within the direct pathway of the basal ganglia, the pathway that facilitates movement, were specifically upregulated, supports the use of ECT to treat the motor symptoms of PD.

In recent years, the development of treatments for PD involving the administration of exogenous neurotrophic factors has been a major focus of research, from basic science to clinical trials. Several different delivery methods have been investigated, including gene delivery via viral vectors (Klein et al., 1999; Bjorklund et al., 2000; Wang et al., 2002; Eslamboli et al., 2005) and direct infusion of the protein into the target area (e.g. intraputamenal infusion of GDNF; (Gill et al., 2003; Patel et al., 2005; Slevin et al., 2005; Lang et al., 2006). However, this line of investigation faces significant barriers because of the large size of the proteins, and their inability to cross the blood-brain-barrier, and hence the necessary invasiveness of the treatment. Currently, safety concerns are prohibiting the use of viral vectors in human patients, and one Phase II GDNF direct infusion study was halted due to a lack of statistically significant improvement and the appearance of neutralizing antibodies in several patients (Lang et al., 2006). While the therapeutic potential of neurotrophic factors in treating PD and other diseases is well recognized, clearly much more work must be done before their use is a clinical reality.

In contrast, ECT treatment may be a non-invasive method of delivering endogenously produced neurotrophic factors to the brain regions that lose their normal trophic support in parkinsonism. If, as our data suggest, part of the mechanism of action of ECT in PD is the enhancement of striatal BDNF and FGF-2 concentrations, then ECT may provide symptomatic relief for PD patients by either limiting cell death or promoting cell growth.

ECT may be a viable therapeutic option for several specific populations of PD patients. Since ECT is primarily used as a treatment for depression, and since 30-40% of PD patients are also depressed (Tandberg et al., 1996; Slaughter et al., 2001), this seems like an ideal population to treat with ECT. If the neurotrophic effects of ECS are one of the prime outcomes, then patients in the early stages of the disease may benefit the most, since they will still have some remaining DA terminals. Neurotrophic factors could enhance the function of remaining DA terminals either by promoting their survival or by stimulating growth, perhaps in the form of sprouting, to allow them to compensate for those DA neurons that have already been lost. ECT should also be tried in patients who have severe motor symptoms and/or are experiencing a poor response to pharmacotherapy. The usual course of action in such patients is surgery for PD, in the form of pallidotomy or DBS. But brain surgery has inherent risks, and many older patients
are not good surgical candidates. Before undergoing surgery, these patients should consider a course of ECT. ECT may also be worth considering in patients with young onset PD, since this population typically experiences complications of L-DOPA treatment much earlier, and delaying L-DOPA treatment for as long as possible is recommended (Kostic et al., 1991).

Conclusions:

In conclusion, this body of work describes a series of studies undertaken to investigate the mechanism of action of ECT in PD. After carefully evaluating our animal models and developing a set of tools to use, we have shown that repeated ECS treatment in parkinsonian animals has specific effects on motor behaviour, and on striatal DA receptors and neurotrophic factor levels. The combined DAergic and neurotrophic effects of ECS treatment may account for the improved motor function after ECT treatment in PD patients. ECT is a safe, effective, and widely available treatment for depression, and our studies, showing similar effects in the striatum in the parkinsonian brain as the effects in limbic brain regions used to explain the mechanism of action of ECT in depression, provide support for its further use and study as a potential adjunct treatment for PD.
References:


Metz A, Heal DJ (1986) In mice repeated administration of electroconvulsive shock or desmethylimipramine produces rapid alterations in 5-HT2-mediated head-twitch responses and cortical 5-HT2 receptor number. Eur J Pharmacol 126: 159-162.


Smith SE, Sharp T (1997) Evidence that the enhancement of dopamine function by repeated electroconvulsive shock requires concomitant activation of D1-like and D2-like dopamine receptors. Psychopharmacology 133: 77-84.


Yates M, Leake A, Candy JM, Fairbairn AF, McKeith IG, Ferrier IN (1990) 5HT2 receptor changes in major depression. Biol Psychiatry 27: 489-496.


THE USE OF ANAESTHESIA FOR REPEATED ECS TREATMENT IN THE RAT

Introduction:

Electroconvulsive shock (ECS) is a widely used animal model of the application of electroconvulsive therapy (ECT) to human patients as a treatment for psychiatric disorders. There is a major discrepancy, however, between the method of administration of ECS to rodents vs. ECT to humans, in that rodents usually receive ECS without anaesthesia, whereas in modern ECT, humans are always anaesthetized. In developing our protocol for the administration of ECS to rats, we wanted to model the clinical situation as closely as possible, and chose to administer ECS under anaesthesia. To investigate the use of anaesthesia for ECS, we administered a course of ECS treatment under three different anaesthetics, ketamine hydrochloride, a non-competitive NMDA antagonist dissociant, thiopental sodium, a short-acting barbiturate, and isoflurane gas, a volatile anaesthetic. We chose the three anaesthetics based on their applicability to clinical ECT (ketamine and thiopental are used routinely in humans), and their ease of use (isoflurane is a safe and widely used gas anaesthetic). Using a small number of subjects in each group, we examined the feasibility of using these anaesthetics, and their effects on seizure threshold and duration compared to ECS without anaesthesia.

One of the most consistently reported effects of repeated ECS in rodents is increased 5-HT2 receptor binding and mRNA in the frontal cortex (Kellar et al., 1981; Vetulani et al., 1981; Green et al., 1983; Vetulani et al., 1983; Stockmeier and Kellar, 1986; Pandey et al., 1992; Butler et al., 1993; Burnet et al., 1999). Interestingly, this effect appears to be opposite to the effects of successful antidepressant drug treatment in human patients (Attar-Levy et al., 1999; Yatham et al., 1999; Meyer et al., 2001; Mischoulon et al., 2002) and repeated ECS in non-human primates (Strome et al., 2005a). In rodents, too, chronic treatment with tricyclic antidepressants (TCAs) has repeatedly been shown to decrease cortical 5-HT2 receptor binding (Cross and Horton, 1988; Newman et al., 1990; Peroutka and Snyder, 1990; Goodnough and Baker, 1994; Strome et al., 2005b). Although it has long been known that ECS in rodents has opposite effects on 5-HT2 receptors, compared to the effects of most antidepressant drug treatments in rodents and humans, an explanation for this inconsistency has not been presented. One possible explanation may arise from the fact that rodents are typically not anaesthetized during ECS treatment. This lack of anaesthesia may impact the immediate effects of ECS, thereby influencing the downstream compensatory changes that accrue with repeated treatments.

After evaluating the feasibility of using anaesthesia for ECS, we chose ketamine as our anaesthetic of choice for all further studies. To support our decision to use ketamine, and to further corroborate our in vitro autoradiographic technique described in Chapter 3, we also examined frontal cortex 5-HT2 receptor binding in animals treated with ECS or sham under ketamine.

Materials and Methods:

Adult male Sprague-Dawley rats were the subjects of this experiment. Subjects (n = 4-10 per group) were given ECS as described in Chapters 5 and 6 every day for 10 days, under one of four conditions: 1) ketamine HCl (80 mg/kg i.p.), 2) thiopental sodium (20 mg/kg i.v.), 3) isoflurane (4% in O₂ as needed), or 4) without anaesthesia, hereafter referred to as the KET,
THIO, ISO and NO groups, respectively. Control rats were given the same treatment and electrodes were placed, but no current was administered.

Animals in the KET group were sacrificed by decapitation 24 hours after the last treatment. The brains were removed and quickly frozen in isopentane cooled with dry ice, and stored at -80°C until sectioning. Twenty micron coronal sections were cut, thaw-mounted onto microscope slides, and the slides were stored at -80°C until the receptor binding assay was performed. Serotonin 5-HT\textsubscript{2} receptor binding with \textsuperscript{[18]}F]setoperone was performed on sections through the frontal cortex as described in (Strome et al., 2005b).

Results:

Effects of Anaesthesia and ECS on Body Weight

The mean weight of each group was not significantly different, nor was there a significant effect of ECS on weight in any of the groups. All groups were significantly heavier at the end of the course of ECS compared to the beginning (Two-way repeated measures ANOVA: Main effect of time, \(F_{1,66} = 8.16, p < 0.01\); data not shown).

Stimulus Properties and Seizure Characteristics

Due to the difficulty of finding an adequate tail vein to inject into each day, the THIO group did not receive ECS every day for 10 days. Animals in both the sham and ECS conditions did, however, receive at least 8 treatments, with at least the last 4 treatments administered on consecutive days, and no animal ever went more than 2 days without treatment.

Figure A.1 shows the relationship between the stimulus intensity and the course of treatment under all four conditions. In order to continue to elicit similar length seizures in the anaesthetized groups, stimulus intensity (charge dose: \#mC administered = stimulus duration x current x pulse width x frequency) was increased as the course of treatment progressed. This increase was significant in the KET, ISO and THIO groups (linear regression, F-test for slopes, \(p < 0.05\) for all), but not in the NO group (\(p > 0.05\)) (Fig. A.1). The slopes of all four lines were significantly different, but the mean charge doses were not significantly different between the KET and THIO groups (Tukey test, \(p > 0.05\)).

Seizure length (mean 9-12 s for anaesthetic groups, 75 s for non-anaesthetized) did not change significantly as the course of ECS progressed (linear regression, F-test for slopes, \(p > 0.05\) for all), but animals in the NO group had significantly longer seizures than all other groups (Fig. A.2; ANCOVA for intercepts: \(F_{3,35} = 646.35, p < 0.01\)).

All of the animals in this study experienced a motor seizure after receiving the ECS stimulus each day, however not every animal experienced a fully generalized seizure with tonic hind limb extension (THLE) after each ECS treatment. We have adopted the terminology of (Andrade et al., 2002)and colleagues (2002) to describe the types of motor seizures elicited after ECS in rats. In the KET and THIO groups, the probability of eliciting a completely generalized seizure with THLE (Type 3 seizure) increased as the course of ECS progressed (Fig. A.3). In the NO group, the animals invariably exhibited THLE, whereas animals in the ISO group usually did not exhibit THLE as part of the motor seizure (Fig. A.3). All seizures without THLE were similar to the Type 2 seizures described by Andrade and colleagues (2002) i.e. tonic-clonic seizures involving mainly the head and forelimbs. Although we did not measure it directly, the recovery from the seizure (the time it took for the animals to move around spontaneously with good balance and coordination) was about the same for all three anaesthesia groups, but was much faster for the animals treated without anaesthesia.
Figure A.1: Mean charge dose (± SEM) required to elicit a seizure during the course of ECS under one of four conditions.

Figure A.2: Mean seizure length (± SEM) during the course of ECS under one of four conditions.
5-HT$_2$ Receptor Binding in Ketamine-Treated Animals

Repeated ECS treatment under ketamine anaesthesia significantly increased 5-HT$_2$ receptor binding in the frontal cortex (Student’s t-test: $t = 3.08$, $p < 0.008$; Figs. A.4, A.5).

Figure A.3: Probability of eliciting a seizure with tonic hindlimb extension (THLE) under four different conditions as the course of ECS progressed.

Figure A.4: Repeated ECS treatment under ketamine increases frontal cortex $[^{18}$F]$setoperone$ binding, ($^*$ $p < 0.05$).
Figure A.5: Frontal cortex 5-HT$_2$ receptor binding in rats treated with ECS or sham under ketamine anaesthesia as measured by [$^{18}$F]setoperone.
Discussion:

In this study, we evaluated the use of three different anaesthetics for repeated ECS treatment in rodents, compared to the more common method of administering ECS to these animals without anaesthesia. We found that, in general, the use of anaesthesia for ECS administration influences the seizure characteristics, requiring higher charge doses to continue to elicit similar length seizures as the course of treatment progressed (i.e. increased seizure threshold), and decreasing both seizure length, and the probability of eliciting a fully generalized seizure.

In addition, there were feasibility and applicability issues of using anaesthesia for repeated ECS in rodents. Thiopental is a short-acting barbiturate, the class of anaesthetic most commonly used for human ECT (Wagner et al., 2005). However, these drugs require intravenous administration, which was difficult to accomplish non-invasively on a daily basis in the rat. Isoflurane is a widely used volatile anaesthetic, but it has anticonvulsant properties (Eger, 1985), and is generally not used for ECT, and the subjects in this group therefore had a low probability of a fully-generalized seizure (Fig. A.3). Based on these factors, its relatively mild anticonvulsant effects, and the fact that it is commonly used for human ECT, we chose ketamine as our anaesthetic of choice for all future studies.

We also examined frontal cortex 5-HT$_2$ receptor binding in the KET group, and, as in many other reports, found that repeated ECS treatment increases binding to this receptor. While this upregulation of 5-HT$_2$ receptors is unexpected based on the effects of antidepressant drugs in humans and animals and repeated ECS in non-human primates, it may be explained by technical differences in the administration of the treatment (differences in schedule, current, or electrode placement), or by species differences in metabolism, neuroanatomy, or neurochemistry.

In conclusion, we have shown here the feasibility of using ketamine anaesthesia for repeated ECS in rats, and that, as in ECS in rodents without anaesthesia, ECS under ketamine upregulates frontal cortex 5-HT$_2$ receptor binding.
References:


THE EFFECTS OF REPEATED ECS UNDER KETAMINE ANAESTHESIA ON BDNF mRNA EXPRESSION

Introduction:
One of the most widely reported effects of repeated electroconvulsive shock (ECS) on the rodent brain is the upregulation of brain-derived neurotrophic factor (BDNF) mRNA expression in limbic regions (Nibuya et al., 1995; Smith et al., 1997; Zetterstrom et al., 1998; Dias et al., 2003; Jacobsen and Mork, 2004). However, most studies of the effects of repeated ECS treatment in rodents are performed without anaesthesia, whereas we have opted to use ketamine anaesthesia in our model. In addition, no reports have been made on the effects of repeated ECS treatment on BDNF mRNA expression in the striatum, our primary region of interest in examining the mechanism of action of electroconvulsive therapy in Parkinson’s disease. In this study, we investigated the effects of repeated ECS treatment under ketamine anaesthesia on BDNF mRNA expression in the piriform cortex, a limbic brain region, and striatum of unilateral 6-hydroxydopamine (6-OHDA)-lesioned rats.

Materials and Methods:
Repeated ECS or sham treatment (n = 7 per group) was administered under ketamine anaesthesia to unilateral 6-OHDA-lesioned rats as described in Chapters 5 and 6. Twenty-four hours after the last treatment, the animals were sacrificed by decapitation, their brains frozen in isopentane cooled with dry ice, and stored in a -80°C freezer until sectioning. Twenty micron sections were thaw-mounted onto glass slides in pairs, with one section from a sham-treated and one section from an ECS-treated animal per slide, alternating the placement on the slide of sham- and ECS-treated animals (top or bottom). In situ hybridization for BDNF mRNA was performed as described by (Kobayashi et al., 1996).

Results:
We were unable to detect any hybridization signal in the striatum. In the piriform cortex, ECS treatment significantly increased BDNF mRNA expression compared to sham-treated controls (Unpaired T-test, $t_{12} = 2.82$, p < 0.02; Fig. B.1).

Discussion:
As in previous reports (Hofer et al., 1990; Maisonpierre et al., 1990) no hybridization signal was detected in the striatum of either ECS- or sham-treated rats. Protein levels of BDNF in the striatum are detectable, and we (Strome et al., 2006) and others (Angelucci et al., 2002; Altar et al., 2003) have shown that striatal BDNF protein increases after repeated ECS treatment. The observation that BDNF protein is present in the striatum, while mRNA is absent has led to the suggestion that striatal BDNF is anterogradely transported from other brain regions, and Altar and colleagues have shown that, indeed, this is the case, with most striatal BDNF arising from frontal cortical projections, and a small amount arising from nigrostriatal projections (Altar et al., 1997).

In this study, we also found that, as in animals administered ECS without anaesthesia, ECS under ketamine anaesthesia increases BDNF mRNA within the piriform cortex. This brain
region is part of the limbic system and while it has traditionally been thought of as a sensory region, based on its connectivity with the olfactory system, more recent studies suggest that it functions more as associative cortex, rather than primary sensory cortex (Johnson et al., 2000). One of the most interesting features of the piriform cortex is the fact that it is the only site in the rat brain where small amounts of γ-aminobutyric acid (GABA) antagonists can elicit tonic-clonic seizures (Gale et al., 1992), and this discovery has led the area to be investigated thoroughly for its role in seizures, kindling, and ECS.

In conclusion, this investigation showed that one of the typical effects of repeated ECS treatment on BDNF mRNA expression, its upregulation in the piriform cortex, is not negatively impacted by the use of ketamine as an anaesthetic for ECS. In addition, we identified that BDNF mRNA expression was not detectable in the striatum of unilateral 6-OHDA-lesioned rats, after either ECS or sham treatment.

Figure B.1: The effects of repeated ECS treatment under ketamine anaesthesia on BDNF mRNA expression in the piriform cortex. ECS treatment significantly increased BDNF mRNA compared to sham (*p < 0.02)
References:


