THE LONGITUDINAL EFFECT OF ELECTROCONVULSIVE THERAPY IN THE
RODENT MODEL OF PARKINSON'S DISEASE

by

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Abstract

Electroconvulsive therapy (ECT) is a safe, non-invasive treatment that is widely used to treat psychiatric disorders. Clinical findings suggest that ECT also has positive effects in Parkinson’s disease (PD), and show variable length improvements in motor symptoms. Currently, only acute studies have been performed in 6-OHDA lesioned rats to investigate the effects of Electroconvulsive shock (ECS), showing significant improvements in gross motor function and enhanced striatal D1 and D3 receptor binding, 2 days following treatment.

In this study, we hypothesized that there are persistent effects of ECS in 6-OHDA rats with 1) improved motor behaviour and 2) enhanced D1 receptor binding in the striatum. A multidisciplinary approach combining both behavioural tests and autoradiography was used to investigate the persistence of ECS. 6-OHDA lesioned rats were randomly divided into ECS or sham treatment groups. Motor behaviour was assessed using two non-pharmacological behavioural tests, the tail flick test to evaluate spontaneous rotational behaviour and the tapered ledged beam-walking test to examine hind limb dysfunction. Animals were sacrificed at 2, 14 and 28 days following the treatments, and their brains were processed to determine dopamine receptor binding.

In conclusion, compared to sham-treated animals, ECS treatment significantly decreased spontaneous rotational behaviour and improved hind limb function after 2 and 14 days post-treatment, with return to baseline values at 28 days. However, in contrast to earlier studies, ECS treatment had no apparent effect on striatal D1 receptor binding at any of the three time points. The possible reason for this discrepancy will be discussed in detail. To the best of our knowledge, this study provides the first comprehensive investigation of the longitudinal effects of repeated ECS treatment in the 6-OHDA lesioned rats. ECS-treated animals showed prolonged motor improvements lasting at least two weeks. This study provides further understanding of the longitudinal effects of ECT and its potential use as an adjunct treatment for PD.
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<tr>
<td>5-HT</td>
<td>5-hydroxytryptamine (serotonin)</td>
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<td>6-OHDA</td>
<td>6-hydroxypapamine</td>
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<td>F-DOPA</td>
<td>[F]fluorodopa</td>
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<td>AADC</td>
<td>aromatic L-amino decarboxylase</td>
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<td>ANOVA</td>
<td>analysis of variance</td>
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<td>ARG</td>
<td>autoradiography</td>
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<td>BDNF</td>
<td>brain derived neurotrophic factor</td>
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<td>Beam test</td>
<td>tapered/ledged beam-walking test</td>
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<td>BG</td>
<td>basal ganglia</td>
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<td>cAMP</td>
<td>cyclic adenosine monophosphate</td>
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<td>DA</td>
<td>dopamine</td>
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<td>DBS</td>
<td>deep brain stimulation</td>
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<td>DLU</td>
<td>digital light units</td>
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<td>ECS</td>
<td>electroconvulsive shock</td>
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<td>GDNF</td>
<td>glial cell line-derived neurotrophic factor</td>
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<td>GPi</td>
<td>internal portion of globus pallidus</td>
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<td>GPe</td>
<td>external portion of globus pallidus</td>
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<tr>
<td>HVA</td>
<td>homovanillic acid</td>
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<tr>
<td>i.m.</td>
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<td>i.p.</td>
<td>intraperitoneal</td>
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<td>i.v.</td>
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<td>L-DOPA</td>
<td>levodopa</td>
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<td>MFB</td>
<td>medial forebrain bundle</td>
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<td>MPTP</td>
<td>1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine</td>
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<td>nucleus accumbens</td>
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<tr>
<td>PD</td>
<td>Parkinson’s disease</td>
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<tr>
<td>PET</td>
<td>positron emission tomography</td>
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<td>ROI</td>
<td>region of interest</td>
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<td>SA</td>
<td>specific activity</td>
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<td>s.c.</td>
<td>subcutaneous</td>
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<td>SN</td>
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<td>substantia nigra pars compacta</td>
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<td>SNr</td>
<td>substantia nigra pars reticulata</td>
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<td>STN</td>
<td>subthalamic nucleus</td>
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<tr>
<td>t½</td>
<td>half-life</td>
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<td>TH</td>
<td>tyrosine hydroxylase</td>
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<td>THLE</td>
<td>tonic hind limb extension</td>
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Chapter 1: Introduction

1.1 Introduction to Research

Parkinson’s disease (PD) is a chronic, progressive neuro-degenerative disorder that currently affects over 100,000 Canadians. The four cardinal symptoms include tremor, muscle rigidity, slowness of physical movement (bradykinesia) and balance and gait abnormalities. These symptoms are primarily resulting from the degeneration of dopamine (DA) containing neurons in the substantia nigra (SN) and depletion of DA in the striatum. The routine treatment option for PD is replacement of the missing transmitter through oral administration of a combination of its direct precursor (L-DOPA, levodopa) with a peripheral dopa decarboxylase inhibitor such as carbidopa, with or without adjuncts of DA agonists. The negative side effects and diminishing effectiveness of levodopa therapy after prolonged use limit its usefulness in advanced PD patients. As a result, surgical interventions such as transplantation of fetal cells and/or deep brain stimulations are performed more commonly but the risks both from surgery and the treatment itself is of concern and limits their use in early patients. The risks, side effects and short-lived improvements associated with some of these treatments have prompted a continued search for alternate methods of treatment, especially in patients which co-morbid depression and mood disorders already reduce treatment options.

Electroconvulsive therapy (ECT) is a widely used and effective treatment for psychiatric and mood disorders. Not only is ECT an effective treatment for depression and psychotic disorders, it also appears to have positive effects in PD, improving motor symptoms for
several weeks. ECT involves administration of electric shocks to induce short-term (20 – 30 seconds) generalized seizures. The risks associated with ECT are minimal, but the treatment is typically associated with some transient memory loss. Studies with ECT in depression show consistent increased activity in the serotonin and DA system via change in the concentration of these neurotransmitters and/or their receptors. In addition, more recent studies suggest increased neurotrophic activity in various brain regions. However, in general, little is known of the central mechanisms of action of ECT in PD.

In animals, repeated treatment with electroconvulsive shock (ECS) has been shown to improve both monoamine neurotransmission and neurotrophic factor concentrations in limbic brain regions (hippocampus mostly) in normal rats and models of depression. Pilot studies were performed in our lab to investigate the existence of similar acute effects of repeated ECS in striatal regions in an animal model of PD. These studies showed that repeated ECS treatment in unilateral 6-hydroxydopamine (6-OHDA) lesioned rats led to acute (24-48hrs) improvements in gross motor function and specific effects on the expression of DA receptors, enhancing striatal D₁ and D₃ receptor binding in the striatum. These preliminary encouraging studies were however only performed at one time post ECS in a small sample size and the persistence of these effects is unknown.

The main focus of this work is based on these pilot observations and is highly relevant to the clinical situation evaluating the central effects and mechanism of action of ECS in PD. Indeed, as a large percentage of PD patients also present with depressive symptoms, often untreated or treated with poor outcomes, ECT, with both its favourable safety profile in
elderly subjects and its anti-depressant and anti-parkinsonian effects may present a number of advantages over alternate anti-depressant therapies.

Using the 6-OHDA rodent model of PD, the longitudinal effects of ECS on motor function and the DA system were investigated. Based on the clinical observations, literature and our previous studies, I hypothesize that:

1. There will be persistence of behavioural improvements after ECS treatment for days to weeks.
2. There will be a parallel persistence of changes in dopamine function as evidenced by up-regulation of D1 receptors in the striatum.

1.2 Parkinson’s Disease

PD is a chronic, progressive neurodegenerative disease that affects more than 6 million people worldwide and approximately 125 per 100,000 Canadians (Lai et al., 2003). The occurrence of PD is rare before the age of 50 but the rate of incidence increases to 1% of the population over the age of 60 and to 4% by age 85. The average age of onset of PD worldwide has been found to be around 60 – 65 years. PD is more commonly diagnosed in men but there is no predisposition regarding ethnicity or geographic location (Samii et al., 2004).

PD was recognized as a medical condition in 1817, when a London physician, James Parkinson published a detailed medical essay, “An Essay on the Shaking Palsy” (Critchley M., 1955). His essay was based on the observed similar clinical feature of 6 patients, where the symptoms were described as “involuntary tremulous motion, with lessened muscular
power, in parts not in action and even when evaporated; with a propensity to bend the trunk forwards, and to pass from a walking to a running pace: the senses and intellect being injured" (Critchley M., 1955). In 1860s, French neurologist Jean Martin Charcot added symptoms such as mask-like facial expression and stooped posture and gave the name of PD (Goetz et al., 1987). Since then, PD has been extensively investigated and studied; yet much remains a mystery.

The degenerative symptoms of PD are understood as a result of dopamine (DA) deficiency in the brain, especially in SN and the striatum. Most patients who are diagnosed with PD have lost 80% or more of their DA producing cells when the symptoms appear (Riederer and Wuketich, 1976; Seeman and Niznik, 1990; Koller, 1992). The chemical differences in the brain of PD patients were identified in 1960s, where low levels of DA were discovered to be caused by the degeneration of dopaminergic cells in SN. This discovery leads to development of the first effective medical treatment for PD, the drug levodopa (L-DOPA) as the first line of medication for treating symptoms. The function of L-DOPA and other treatment options of PD will be described later in this thesis.

1.3 Clinical Features of Parkinson's disease

PD is the second most common neurodegenerative disorder with debilitating symptoms. Idiopathic PD is the most observed form and its manifestation has been characterized as asymmetrical and progressive (Hughes et al., 1993). Patients with idiopathic PD will develop several symptoms over time, but they all typically develop the following symptoms: tremor, rigidity, slowness of movement (bradykinesia), postural instability and difficulty in walking (parkinsonian gait) (Calne et al., 1992; Bernheimer et al., 1973). Since the definite
diagnosis of PD can be done only through autopsy, physicians require three primary symptoms (tremor, rigidity and bradykinesia) to make the clinical diagnosis (Sethi, K., 2003). Postural instability and gait disturbance emerges at a later stage of PD.

Although motor dysfunction is the hallmark of PD, there are non-motor symptoms that are associated with PD. Some of these non-motor symptoms are common and potentially disabling manifestation of PD. For example, there are psychiatric impairments such as depression, dementia, anxiety, psychosis and memory loss as well as autonomic dysfunction such as problems with gastrointestinal, cardiovascular and thermoregulatory functions (Marsh, 2000; Leentjens, 2004). The most observed psychiatric problem in PD is depression, evident in approximately 40% of PD patients (Marsh, 2000; Cummings, 1992; Slaughter et al., 2001).

1.4 The Basal Ganglia: Functional Organization and Pathophysiology of PD

1.4.1 Neurotransmitter Dopamine

Dopamine (DA) is a catecholamine that has an important role in the neural system in motor activity regulation, cognition and behaviour, and motivation and reward system (Volkow et al., 1996; Le Moal and Simon, 1991). Abnormities in the brain DA signalling are associated with a number of neurodegenerative diseases such as PD, Alzheimer’s disease and psychiatric disorders such as schizophrenia (Calne et al., 1992; Volkow et al., 1999; Werkman et al., 2006; Malhi and Berk, 2007). In PD, DA-producing neurons in the SN are destroyed with reduced DA level in the striatum.
DA acts as a neurotransmitter in the brain and is synthesized by the dopaminergic neurons of the central nervous system. The amino acid tyrosine is converted to dihydroxyphenylalanine (L-DOPA) by tyrosine hydroxylase, the rate-limiting step in the synthesis of DA (Nagatsu et al., 1964). L-DOPA is then subsequently converted to DA by the aromatic L-amino acid decarboxylase (AADC). Please refer to Figure 1 for the dopamine biosynthesis.

Dopaminergic cells are mostly located in the midbrain in SN, ventral tegmental area (VTA) and retrobulbar areas. In PD, the nigrostriatal DA system that is associated with the initiation and execution of movement is mainly affected. Therefore, for the purposes of this study, this thesis will focus on the alteration of dopaminergic in the nigrostriatal pathway.

1.4.2 The Basal Ganglia System

DA functions largely in the movement-associated nigrostriatal pathway, a part of the basal ganglia (BG). The movement is regulated by a set of feedback loops that involves the cerebral cortex, the thalamus and the basal ganglia. The BG are a set of sub-cortical nuclei that are located deep in the brain, beneath the cerebral cortex and surround the thalamus and the hypothalamus.
The BG are an integral part of the motor circuits and participate in the modulation and facilitation of movements (Alexander and Crutcher, 1990). In addition, they are also associated with non-motor activities such as memory, emotion and cognitive functions (Graybiel et al., 1994; Parent and Hazrati, 1995). For the purpose of this thesis, the following discussion will only focus on the motor function of the BG circuit. Extensive research has been carried out to explain the mechanisms by which the BG influence the motor function, but their role and function remains largely unknown. Further understanding of the structures and function of the nuclei of the BG are required to explain both normal and abnormal motor behaviours.

The BG are located in the forebrain and are composed of interconnected nuclei known as the caudate nucleus, the putamen, the globus pallidus (internal and external segments), the subthalamic nucleus and the substantia nigra (pars compacta and pars reticulata). The striatum is composed of the caudate nucleus and the putamen, which are separated by the white matter tract known as the internal capsule. The striatum is the primary afferent nucleus of the BG and receives inputs mainly from the cortex and the substantia nigra pars compacta (SNc). The primary output structures are the globus pallidus internal (GPi) and substantia nigra pars reticulata (SNr). These two structures have a common embryological origin and contain cytologically similar neurons (Albin et al., 1989).
This thesis used the rodent model of PD to investigate the longitudinal effects of ECS. It is important to note that, although the function of the BG nuclei in humans and rodents are quite similar, there are striking differences in their anatomical positions (Figure 2 and 3). One of the most prominent differences between human and rat BG is the orientation of the caudate and putamen. In humans, the caudate and putamen are separated by an internal capsule. In the sagittal view, the caudate nucleus forms a ‘C’ shaped structure, with a rostral head, caudal body and a tail ventral to the head and body. The putamen is located lateral to the caudate and sits within the ‘C’ structure. Conversely, in rodents, the internal capsule is located medial to the caudate. As a result, there is no clear division between the caudate and putamen in the rodent anatomy. Also, the rodent striatum is a linear structure that moves laterally, without forming a ‘C’ shaped caudate or ventrally extending tail.

Figure 2: The Human Basal Ganglia System
In both anterior and posterior section of the brain, the internal capsule separates the caudate and putamen.
Another difference between human and rodent basal ganglia is the globus pallidus. In humans, the globus pallidus is divided into two segments (external and internal segments) by the internal medullary lamina. While the rodent GPe is analogous to the human GPe, the GPi structure in human is non-existent in rat. Instead, the entopeduncular nuclei in rats are thought to be performing functions that are carried out by the GPi in humans. Despite the aforementioned anatomical discrepancies between human and rodent basal ganglia, the rodent model was used for this thesis, as it is a widely accepted and used animal model for PD.

Figure 3: The Rodent Basal Ganglia
This diagram is showing the anterior and posterior sections of the striatum. Note that the caudate and putamen are not separated by the internal capsule in rats.

1.4.3 The Direct and the Indirect Pathway

The basal ganglia participate in a parallel circuit from cerebral cortex to thalamus and back to cortex in a cortico-basal ganglia-thalamo-cortical loop (Parent et al., 2000). This BG motor circuit is involved in planning and production of movement. Voluntary movement is mediated through two independent and opposite pathways known as the direct and the
indirect pathways (Alexander and Crutcher, 1990; Parent and Hazrati, 1995; Albin et al., 1989; Mink, 1996).

In the direct pathway, glutameric cortical stimulation excites striatal GABAergic neurons. These neurons send monosynaptic GABAergic inhibitory projections to output neurons in the GPi and the SNr. Normally, GPi and SNr neurons project to and inhibit ventrolateral nuclei of the thalamus. Due to inhibition of GPi and SNr, the thalamus receives less inhibition. Thus, when a particular movement is selected, the appropriate thalamic neurons get disinhibited and facilitate in execution of planned movement. The indirect pathway also sends glutameric cortical projection and excites striatal GABAergic neurons. However, these neurons send inhibitory projection to the GPe, resulting in decreased inhibitory input to STN. Disinhibition of STN leads to increased glutameric excitatory stimulation to the GPi and the SNr. Activation of GPi and SNr increases inhibition of thalamus, which decreases glutameric excitatory input to the cortex. Therefore, the indirect pathway functions to suppress unwanted motor behaviour. In summary, glutameric cortical activation of the direct and the indirect pathway has opposing effect on the execution of movement. For example, when flexing a muscle, the direct pathway activates flexor whereas the indirect pathway inhibits contractor. (Albin et al., 1989; Alexander and Crutcher, 1990; Gerfen, 1992)

Please refer to Figure 4 for the diagram of the direct and the indirect pathway.

DA receptors belong to a class of G-protein coupled receptors and are divided into five different subtypes. There are two classes of receptors on the striatal neurons: D1-like DA receptors that consist of D1 and D5 receptors and D2, D3 and D4 receptors are categorized into D2-like DA receptors (Altar and Marien, 1987; Bouthenet et al., 1987; Jaber et al., 1996).
D1 receptors are able to activate enzyme adenylate cyclase, which increases intracellular concentration of cyclic adenosine monophosphate (cAMP). Increased level of cAMP in neurons typically has excitatory effect. In contrast, D2 receptors increase phosphodiesterase activity, which break down cAMP, producing inhibitory effect in neurons (Jackson and Westlund-Danielsson, 1994; Memo et al., 1986). In the striatum, D1 and D2 receptors are involved in both direct and indirect pathway and play a crucial role in PD manifestation.

In the direct pathway, DA from SNc enhances the activity of the striatal neurons that express D1 receptors. When DA binds to the striatal neurons bearing D1 receptors, these neurons get activated and send inhibitory inputs to the GPi and the SNr. As a result, the thalamus gets disinhibited, leading to facilitation of planned motor program. In the indirect pathway, DA from SNc inhibits the activity of striatal neurons that predominantly express D2 receptors. When DA binds to the striatal neurons bearing D2 receptors, the activity of these neurons decreases. Inhibitory projection to the GPe gets reduced, resulting in increased inhibition of STN. This leads to decreased activation of the GPi and the SNr and the thalamus gets disinhibited. Ultimately, DA functions to facilitate movement in the indirect pathway. DA has a common effect through both direct and indirect pathway; it favours movement by disinhibiting the thalamus. It is important to note that while glutamergic cortical stimulations facilitate motor behaviour via opposing effect of the direct and indirect pathway, DA itself has positive aims at favouring movement in any condition. (Gerfen et al., 1990; DeLong and Wichmann, 2007)
Figure 4: The Normal Basal Ganglia Motor Circuit
Excitatory stimulations are outlined by blue arrow while inhibitory stimulations are represented by black arrow.

1.4.4 Pathophysiology of Parkinson’s disease

Parkinson’s disease is characterized by the progressive depletion of dopaminergic neurons in the substantia nigra (SN) (Obeso et al., 2000; Obeso et al., 2002). Normally, the SN has neurons that contain dark brown pigment called neuromelanin, and these cells are responsible for making the neurotransmitter dopamine. In PD, degeneration of dopaminergic neurons result in abnormally pale appearance of the SN (Hirsch et al., 1988), and this is a hallmark characteristic of PD manifestation.
In PD, the greatest DA deficit is in the lateral part of the striatum, particularly in the putamen (Agid et al., 1989). The degeneration of dopaminergic neurons in the SNC ultimately results in a significantly reduced DA level in the striatum, which leads to increased neuronal activity of the GPi and the SNr. Over-activation of the GPi and the SNr subsequently leads to excessive inhibition of the thalamus and produce increased thalamocortical inhibition. Although, the specific mechanisms of PD symptoms are unclear, it is widely accepted that DA neuronal loss in SNC affects the direct and indirect pathway (Figure 6).

In the direct pathway, DA deficiency reduces the activity of striatal GABAergic neurons. This leads to decreased inhibition of the GPi and SNr, which results in increased inhibitory action on the thalamus. In contrast, DA deficiency in the indirect pathway leads to over-inhibition of GPe by the striatal neurons. This results in disinhibition of the STN and subsequently in over-activation of the GPi and the SNr. In summary, both pathways

Figure 5: Comparison of substantia nigra between Parkinsonian and normal brain. This midbrain sections show the loss of pigmented cells in substantia nigra in Parkinson's disease.
contribute to excessive inhibition of thalamus and therefore, decreased motor activity (Wichmann and DeLong, 1993).

Figure 6: The Basal Ganglia Motor Circuit in PD
Note that in PD, the lack of DA producing cells result in the decrease in inhibitory striatal innervation to GPi and SNr neurons (the direct pathway). In the indirect pathway, there is increased inhibitory striatal innervation to the GPe, which lead to decreased inhibition of STN. Due to disinhibition of STN, there is increased excitatory stimulation to the GPi and SNr. The net result is increased inhibitory projection to the thalamus leading to decreased motor activity.
1.5 Treatment Options in Parkinson’s disease

1.5.1 Drug Therapy: Levodopa (L-DOPA)

L-DOPA is a precursor to DA, which gets converted to DA by the aromatic L-amino acid decarboxylase (AADC). It was first discovered in 1960s, and is the most effective treatment for PD symptoms to this day. L-DOPA therapy is the “gold standard” against which new treatments and therapies are compared (Blandini and Greenamyre, 1999; Olanow et al., 2004). L-DOPA provides significant symptomatic improvements in early PD patients.

L-DOPA is used due to its ability to cross the blood-brain barrier whereas DA itself cannot. When L-DOPA is taken orally, it gets absorbed through the lumen of the small intestine and into the blood. L-DOPA must pass through the blood-brain barrier, and upon entrance into the central nervous system it is metabolized to dopamine via AADC. Due to large amount of AADC present in intestinal walls, liver, kidney and brain endothelium only about 1% of the orally ingested L-DOPA enters the brain to be decarboxylated (Mannisto and Kaakkola, 1990). In order to circumvent this problem, addition of AADC inhibitors such as carbidopa or benserazide is required to enhance the effectiveness and efficiency of the L-DOPA treatment.

L-DOPA is an effective treatment option for early parkinsonian symptoms as it improves motor deficits and extends life expectancy. However, motor complications and symptomatic deterioration develop in 50 – 80% of PD patients within 5 years of initiation of L-DOPA treatment (Marsden and Parkes, 1977; Rajput et al., 2002). The exact cause of motor complications is unclear but evidences suggest that they are related to combinations of pulsatile stimulation of DA post-synaptic receptors possibly leading to functional alterations
of these receptors, changes in DA turnover and likely, changes in downstream pathways (Olanow et al., 2004). Example of treatment complications includes: L-DOPA induced dyskinesias (involuntary movements of the head, trunk and limbs), “wearing-off” (shortened L-DOPA-induced improvements and rapid recurrence of parkinsonian symptoms in between L-DOPA doses) and sometimes psychoses. L-DOPA has its limitations as a long-term PD therapy and new treatment options should be investigated.

In addition, DA agonists are frequently used in combination with L-DOPA or taken alone to provide symptomatic benefit in early stages of PD (Kondo, 2002). DA agonists exert antiparkinsonian effects by acting directly on post-synaptic DA receptors. They were introduced as an adjunct treatment to L-DOPA therapy to reduce L-DOPA associated motor complications and dyskinesia (Hagan et al., 1997). One of the main advantages of DA agonists use is that they do not undergo oxidative metabolism, and they do not form cytotoxic free radicals that may be associated with neuronal degeneration (Szczudlik and Rudzinska, 2007). However, DA agonist therapy has its limitation and the symptoms cannot be adequately maintained by monotherapy alone after few years and patients require L-DOPA to sustain good benefit (Ogawa, 1998).

1.5.2 Cell Therapy – Neural Transplantation

Due to the negative side effects of chronic L-DOPA use, other therapies have been extensively investigated. The pathophysiology of PD has been anatomically well defined and the structures involved in the motor pathways are surgically accessible. Furthermore, as grossly only one neurotransmitter is affected, neural transplantation of specific DA-
producing cells is a suitable alternative for treating PD by providing a long-term and continuous dopaminergic source (Borlongan and Sanberg, 2002).

The most widely studied cell therapy is transplantation of fetal ventral mesencephalic (FVM) cells (Bjorklund and Lindvall, 2000; Olanow et al., 1996). FVM cells implanted in a parkinsonian striatum produce DA, replacing the lost endogenous DA and producing continuous stimulation of the post-synaptic receptors. Patients with FVM cell transplant showed significant clinical improvements and increased striatal dopaminergic activity up to two years post-transplantation, which was observed using \(^{18}F\)-DOPA PET (Sawle et al., 1992; Lindvall et al., 1994). In addition, they demonstrated clinical improvements and reduction of "wearing-off" period and dyskinesia due to chronic use of L-DOPA therapy.

However, there are several concerns associated with FVM cell transplantation. In order to treat a single PD patient, large amount of fetal tissue from multiple embryos are required. Also, the limited storage time of fetal tissue allows for a very narrow time frame for successful transplantation. As with any types of transplantation, patients need to take immuno-suppressant drugs for life.

1.5.3 Surgical Techniques

Investigation of alternative treatment options is essential due to limitations in the benefits and the side effects of L-DOPA treatment and cell therapy. In PD, the GPi and the STN are considered to be over-activated (Obeso et al., 2002; Obeso et al., 2000). Therefore, reduction in either GPi or STN neuronal activities through surgical means may control excessive stimulation.
1.5.3.1 Pallidotomy

Pallidotomy is the process of lesioning of GPi by passage of an electric current. This surgical procedure has been shown to reduce the parkinsonian symptoms such as tremor and rigidity as well as dyskinesia from chronic L-DOPA use (Krack et al., 2000). It is considered for patients with advanced PD for whom the L-DOPA treatment has no positive benefits; rather they have developed severe motor fluctuations. Pallidotomy also allows L-DOPA dosage to be reduced and thus either elimination or reduction of dyskinesias.

Although pallidotomy may be an effective therapeutic surgical procedure, it is not without risks and there are numerous side effects (Laitinen, 2000). There are surgical complications such as infections, seizures and strokes. Patients may develop loss of vision, temporal facial paralysis, loss of sensation and memory. Since pallidotomy requires permanent destruction of the brain structure, it could also lead to severe cognitive and neurological deficits, ultimately causing mental deterioration if the location of the lesion is not perfect. Due to these complications associated with pallidotomy, deep brain stimulation is now the preferred option for patients who are considering surgical treatment for advanced PD.

1.5.3.2 Deep Brain Stimulation

Deep brain stimulation (DBS) is a preferred surgical treatment option for PD treatment. Rather than permanently destroying GPi, in DBS, high frequency stimulators are implanted in hyperactive areas in the brain (GPi or STN) to modulate their activity (Bjarkam et al., 2001; Hammerstad and Hogarth, 2001). Application of high frequency electrical current can directly change brain activity in a controlled manner, by sending continuous electrical pulses to the target area (GPi or STN).
Although the underlying mechanisms of DBS are unclear, the process is believed to block the abnormal firing of STN or GPi neurons leading to improvements in PD motor symptoms. It has been observed that there is reduction of the wearing-off motor and L-dopa induced dyskinesias associated with long-term use of L-DOPA. Similar to pallidotomy, patients can decrease their L-DOPA doses.

DBS is not permanent and it can be turned off or removed if problems emerge. However, there are complications associated with this procedure such as the risks of infection, seizures and stroke. In one study, patients experienced mood changes, language deficit and weakness (Hariz, 2002).

1.5.4 Electroconvulsive Therapy

PD is associated with psychiatric complications such as depression and anxiety. According to the prevalence studies, about 30 – 40% of PD patients suffer from co-morbid major depression (Tandberg et al., 1996; Slaughter et al., 2001). Unfortunately, L-DOPA and/or dopamine agonists do not alleviate depressive symptoms. As a result, patients need to rely on antidepressant drugs, but these drugs may interact negatively with antiparkinsonian medications (Caley, 1997; Gerber and Lynd, 1998).

Electroconvulsive therapy (ECT) is used routinely as a treatment for patients with depression and has also been shown to have beneficial effects on the motor symptoms of PD. ECT was initially administered as a treatment for depression in PD. When ECT was performed on PD patients with co-morbid depression, it not only had positive effects on the mental status but also improved motor disabilities (Kennedy et al., 2003). These observations have been found
to last weeks to months and may last longer with continuous treatment (Aarsland et al., 1997; Fall and Granerus, 1999). In addition, PD patients with wearing-off symptoms showed improvements (Balldin et al., 1981; Andersen et al., 1987). Thus, complete replacement of PD treatment with ECT is unlikely; rather ECT may supplement pre-existing treatment by allowing reduction of the drug doses and improve depression simultaneously.

It is important to note that, although very low, there are some risks associated with ECT. ECT treatment is consistently associated with memory disturbance, where patients experience transient both retrograde and anterograde memory loss (Rami-Gonzalez et al., 2001). However, the loss is temporary and involves only weeks during ECT treatment. ECT has very little contraindications and is a very safe therapeutic alternative.

ECT is believed to affect dopamine functions based on the observation that it induces dopamine-mediated behaviours in animals (Fall et al., 1995a). In animal model of PD, electroconvulsive shock therapy (ECS) has shown a wide rage of effects from anti-parkinsonian motor behaviours to changes in receptor binding, where there is increased responsiveness of dopamine postsynaptic receptors (Strome et al., 2007). In a pilot study, ECS treatment on parkinsonian rodent model showed improvements in motor symptoms as well as up-regulation of dopamine D1 receptors in the striatum at 2 days post-ECS treatment (Strome et al., 2007). This thesis will investigate the longitudinal effects of ECS treatment at 2, 14 and 28 days post ECS treatments.
1.6 Animal Model of PD

Introduction of a catecholamine neurotoxin 6-hydroxydopamine (6-OHDA) in the 1960s enabled researchers to use animal models, rodents in particular, for pre-clinical PD research (Ungerstedt, 1968). 6-OHDA is a selective neurotoxin to dopaminergic and adrenergic cell bodies and causes nerve terminal degeneration (Simola et al., 2007). In the parkinsonian rodent model, 6-OHDA is injected directly into the substantia nigra and medial forebrain bundle to target the dopaminergic pathway (Deumens et al., 2002). The toxin enters the neurons through the DA transporter, leading to a highly selective degeneration of dopaminergic neurons. In order to protect noradrenergic neurons, the noradrenergic transporter blocker desipramine is also co-injected to inhibit the uptake of 6-OHDA by noradrenergic neurons.

Rats with bilateral 6-OHDA lesions most resemble the motor symptoms observed in human PD patients (Tillerson et al., 2001). These rats exhibit clinical symptoms, such as postural instability, reduced capacity to maintain balance and gait disturbance (Cepeda et al., 2007b). However, the bilateral lesions are rarely used due to high morbidity and mortality rates. Therefore, unilateral 6-OHDA lesions are the most widely used lesioning technique. This procedure produce hemi-parkinsonian state where the contralateral side (opposite side of the lesion) of the body is severely affected while the ipsilateral side (same side of the lesion) remains normal, acting as a control. Double injections of 6-OHDA to the SN and MFB result in almost complete loss (>90%) of striatal dopamine level.

As with bilateral lesions, unilateral 6-OHDA lesions lead to prominent asymmetrical motor behaviour. For example, body postural instability, deficit in spontaneous and skilled
movements, as well as sensorimotor difficulties are observed (Barneoud et al., 1995; Kirik et al., 1998). Therefore, behavioural assessments are a useful tool for studying the effectiveness of potential anti-parkinsonian treatments. For example, rats with right unilateral 6-OHDA lesions will turn spontaneously towards the side of the lesion. Increased turning behaviour can be obtained by injection of DA agonists (apomorphine or amphetamine) (Schwarting and Huston, 1996; Kirik et al., 1998; Ungerstedt and Arbuthnott, 1970). However, administration of these drugs may affect the result of some investigations and, in recent years, non-pharmacological tests have become a prominent feature in behavioural assessments. Tapered ledge bean walking test is another behavioural test that measures motor ability of coordinated movements (Strome et al., 2007; Cepeda et al., 2007b; Schallert et al., 2003).

1.7 Autoradiography

Autoradiography (ARG) is an in vitro technique to identify and investigate the mechanism of action of the receptors and transporters in mediating physiological effects. Using post-mortem tissue sections, binding sites can be detected and quantified by the distribution and activity of radiotracers. ARG is particularly useful because it eliminates possible confounding factors commonly observed in vivo techniques such as positron emission tomography (PET) (Laruelle, 2000). For example, competition between the tracer and endogenous ligands makes it difficult to evaluate the distribution and characteristics of the receptors. Incorporation of ARG in PD studies can provide an understanding to the mechanisms of disease progression as well as biochemical evaluation of potential PD
treatments. In particular, in vitro binding studies can be used to identify specific changes in the brain chemistry due to dopamine deficiency and treatments.

In this thesis, ARG was performed with a radioligand incorporating a long half-life isotope ($^3$H: $t_{1/2} = 12.3$ years). Extensive research has been carried out on DA receptors, in particular D1 and D2 receptors, and the most common post-synaptic targets for radioligand. For the purpose of this study, only D1 receptor binding in the striatum was performed. The role of the D1 receptors in PD is not clearly understood, as there are conflicting evidences of receptor regulation, depending on the stage of the disease and severity of the lesions (Buonamici et al., 1986; Marshall et al., 1989). In a pilot study from our lab, up-regulation of D1 receptors in the striatum was observed at 2 days post ECS treatment (Strome et al., 2007). Thus, we evaluated the effects of ECS on the DA D1 receptors in the striatum at different time points after ECS by ARG, using a ligand specific for DA D1 receptor ($^3$H SCH-23390) (Yu et al., 1998).

This thesis studied the effects of ECS in a longitudinal study to examine the persistence of behavioural improvements and the persistence of changes in DA function seen in the pilot study. This study allowed us to gather more pilot data to design further studies aimed at investigating the relationship between changes in DA receptors and improvement in motor function (for example using antisense nucleotide to block D1 expression, use of PET) and better understand the role that the DA D1 receptors play in a variety of behaviours and pathological conditions.
Chapter 2: Methods

2.1 Animal and Surgical Procedure

A total of 72 female Sprague-Dawley rats (Animal Care Centre Breeding Unit, South Campus, UBC) weighing 225 – 250g at the start of the experiments were used. Female rats were selected as a subject rather than male rats due to their smaller body size, which is necessary for effective electroconvulsive shock (ECS) treatment. The animals were housed in Plexiglas cages at constant humidity (55%) and temperature (21°C) on a 12:12h light-dark cycle, with lights off at 12:00 pm. The animals were grouped in pairs and had free access to rat chow and water. The animals were trained and tested with behavioural tests prior to the unilateral 6-hydroxydopamine (6-OHDA) lesions. Two weeks after the lesion, the animals were randomly assigned to two groups: ECS and sham treatment group. The first set of 24 rats were tested and sacrificed at 48 hours, the second set of 24 rats sacrificed at 14 days and the remaining 24 rats were sacrificed at 28 days after the last ECS. Tail flick test and the tapered ledged beam test data were pooled from the entire group of animals. All procedures were approved by the University of British Columbia Committee on Animal Care.

2.2 6-hydroxydopamine Lesioning

The animals were habituated and handled for at least a week before receiving a right unilateral 6-OHDA lesion of the dopamine nigrostriatal pathway. The 6-OHDA was prepared on the morning of the surgery, where the 6-OHDA was dissolved in 0.05% of L (+)-ascorbic acid and 0.9% of saline. It was then stored in the amber glass tube and in the ice bucket to slow down the oxidation from the light. On the day of the surgery, in order to
protect noradrenergic cell terminals, all animals were injected with desipramine hydrochloride (Sigma) 2.5 mg/kg i.p. 30 – 60 minutes prior to 6-OHDA infusions. The animals were then anaesthetized with isoflurane in O₂ with 4% for induction and 1% for maintenance. During the induction of anesthesia, atropine sulfate was administered (0.05 mg/kg s.c.) to decrease respiratory secretion. Once the animals were completely anaesthetized; they were placed into a Kopf stereotaxic head holder with warm heating blanket to keep the animal warm throughout the surgery (Figure 7).

**Figure 7: Surgical procedure set-up**

With the skull flat between lambda and Bregma, the animals received two unilateral stereotaxic infusions of a 2% solution of 6-OHDA hydrobromide (10μg in 4μL 0.05% L (+)-ascorbic acid in saline; Sigma) in each of the two sites along the right medial forebrain bundle. Coordinates of the lesion sites were: Site 1 (directly in the substantia nigra pars compacta) AP: -2.7 mm, ML: -1.8 mm, DV: -8.0 mm all from Bregma; Site 2 (location along
medial forebrain bundle) AP: -4.7 mm (Bregma), ML: -1.5 mm (midline), DV: -7.8 mm (immediately next to the hole) according to Paxinos and Watson (1997). (Figure 8)

Figure 8: Unilateral 6-OHDA lesion sites.
The top diagram corresponds to site 1 lesion of substantia nigra pars compacta and the bottom diagram corresponds to site 2 lesion of a location along medial forebrain bundle.
An infusion cannula attached to a 50μL Hamilton syringe was mounted on a Harvard infusion pump. The rate of infusion was set at 1μL per minute and was infused at a constant rate for four minutes. Following the infusion, the cannula was left in place for additional four minutes to allow 6-OHDA to diffuse. After the surgery, all animals received analgesia (Anafen 2 mg/kg s.c.), antibiotics (Duplocillin 0.1 mg/kg i.m.) to reduce the risk of infection and saline (5 mL s.c.) to prevent dehydration during recovery. All animals were then placed in an incubator to keep warm and monitored until they fully recovered from anesthesia. Animals were given at least 2 weeks after the surgery to recover before administering ECS treatment.

2.3 Behavioral Assessment

All animals were transported in their home cages to the behavior room 30 minutes prior to testing and allowed to habituate. Testing was consistently done during the last two hours of the light cycle (during 10:00 a.m. – 12:00 p.m.) and the first four hours of the dark cycle (during 12:00 p.m. – 4:00 p.m.). To assess the behavioral effects of ECS treatment on a unilateral 6-OHDA rodent model of PD, the testing were done prior to lesion, 2 weeks after the lesion (before ECS treatment) and based on the group, three different times following the last ECS treatment at 2, 14 and 28 days.

2.3.1 Tail Flick Test

The tail flick test counts the number of rotation in rats and correlates the result with the severity of the lesion. In addition, we incorporated this test for post treatment behavior
analysis to investigate the role of ECS treatment in reducing the asymmetry in dopaminergic neurotransmission.

No training was required for the tail flick test because the rotational behavior is a distinctive behavioral feature of the unilateral lesion model that occurs spontaneously. The testing was performed at 2 weeks post lesion, when the lesion is thought to be complete as well as three different time points post treatment. On the testing day, the animal was brought up to the behavior room to habituate for 30 minutes. One animal was removed from the home cage and placed on a flat surface without any distractions, such as a clean tabletop. The animal was grabbed by the tail in the middle and lifted up such a way that the hind limb is lifted while the forelimb is still touching the table. The animal should be able to support itself by using its forelimb so it should not be lifted completely. Making sure that the animal’s hind limb and forelimb is aligned in a vertical position perpendicular to the surface, the number of the rotations the animal make will be noted. The rotation is counted for 15 seconds or until the animal makes 3 full rotations. One complete tail flick test involves 3 consecutive trials with one-minute pause between each trial. When the animal’s hind limb is lifted, the animal should start turning spontaneously towards the lesion side. If the animal does not rotate, the rat is completely lifted off the surface to provoke rotation.

2.3.2 Tapered Ledged Beam-Walking Test (Beam Test)

The tapered ledged beam-walking test (beam test) is sensitive to dopamine function (Strome et al., 2007; Drucker-Colin and Garcia-Hernandez, 1991; Strome et al., 2006) such that it measures gross changes in hind limb use and coordination. This test is slightly modified
form the traditional rat beam walking tests of Schallert and colleagues (Woodlee and Schallert, 2004). Please note Figure 2.3 for the beam test apparatus.

Prior to unilateral 6-OHDA lesions, all animals were trained to walk along a 165 cm long Plexiglas beam. The beam is 6.5 cm wide on the wide end, which gets progressively narrow to 1.5 cm at the narrow end. The beam is positioned 90 cm above the ground and is at an incline of 15 degrees that lead to the rat’s darkened home cage. The surface of the beam is covered in rubber matting to prevent slipping. The 2.5 cm wide Plexiglas ledges were placed 2 cm below along both sides of the beam to provide a support platform for the animals to step on without falling off the beam, when there is a motor deficit (Figure 9). This incorporation of the ledges prevents postural compensations in lesioned animals and allows them to express the motor deficit.

![Figure 9: Tapered Ledged Beam Walking Test Apparatus dimensions](image)

All animals were trained a day or two prior to the first testing day (pre-lesion). Training consist of 10 trials, where the animal was placed on the beam at points further and further
away from the home cage goal until they walk the entire length of the beam. In other words, the animal started with 3 trials at the beginning of the narrow section, then 3 trials at the beginning of the medium sections and finally 4 trials starting at the wide section. Between each trial, the animal remained in the home cage with the lights in the testing room turned off for one minute for reinforcement purposes. The animal only needs one training session in the lifetime as the task is quickly learned.

After the training, all animals were tested prior to lesion for a baseline control, 2 weeks post lesion, and based on the groups, 2, 14 and 28 days after the last ECS treatment. Before testing, each animal was given one “refresher trial” which was not videotaped. One complete beam test involves 5 consecutive trials, which were videotaped from the rear, providing a clear observation of the hind limb and scored at a later date (Figure 10).

Figure 10: General set-up of tapered ledged beam walking test.
Note the home cage covered in dark plastic on the narrow end of the beam.
To limit any possible bias, an investigator, blind to the status of animal's lesion and the treatment groups, scored the beam test results. Scoring involved counting the number of steps and the number of full and half foot-faults (error) in each of the three sections (wide, medium and narrow sections of the beam) for each hind limb in all 5 trials. Taking a step with 3 or 4 toes hanging over the beam is scored as a half error, while completely stepping off the main surface of the beam onto the ledge is scored as a full error (Figure 11). Normal animals made very few errors, if any, in the narrow section of the beam. The data are then entered into a spreadsheet that calculates the errors per steps ratio for each hind limb per section of the beam of all five trials.

Figure 11: Example of an error (full foot fault) and a step.
The left hind limb, circled in red would be scored as a full error because the rat completely steps off the surface onto the ledge. In contrast, the right hind limb, circled in blue would be scored as a step.
2.4 Electroconvulsive Shock Treatment

ECS treatment began after the post-lesion behavior testing. The animals were randomly assigned into either the ECS or Sham treatment groups and were treated daily for 10 consecutive days between 07:00 and 11:00. Before the treatment, the animals were brought up to the behavior room and allowed to habituate for 30 minutes. Atropine sulfate (0.2 mg/kg s.c.) was injected 30 minutes prior to ketamine hydrochloride (80 mg/kg i.p.) injection. Following the induction of ketamine anesthesia, either sham treatment (control group with electrodes placed on the ear but no current passed), or bilateral ECS (80 – 99 mA, 5 – 9.9 s, 70 pulse/s, 0.5 ms pulse width) was administered. Using a small animal ECS machine (Model 57800, Ugo Basile, Italy), the current was applied via ear-clip electrodes coated with electroconductive gel.

All procedure followed the method used by Strome et al. (2006). On the first day of ECS treatment, all animals in ECS treatment group received same initial current doses. Current applications for subsequent treatments were adjusted based on the length and nature of the previous seizure. The length of the seizure was timed with a stopwatch and all ECS-treated animals had a seizure lasting 10 – 25 seconds, where those exhibiting tonic hind limb extension (THLE) portraying generalized seizures were noted. After the treatment, all animals were placed in an incubator to keep warm until they were fully awake.

2.5 Post-Mortem Studies

2.5.1 Post-Mortem Processing

Based on the groups, the time points for sacrifice were different: subsets of both sham and ECS animals were sacrificed at 2, 14, and 28 days after the last ECS treatment. The brain
was rapidly removed and frozen in isopentane cooled to $-70^\circ$C in dry ice. The brains were then stored at $-80^\circ$C until sectioning.

2.5.2 Post-Mortem Studies: Autoradiography

Using a cryostat (Leica), the brains were sliced in twenty-micron sections at $-21^\circ$C. Serial sections were taken throughout the striatum and were thaw-mounted onto Superfrost Plus glass slides (Fisher Scientific). The tissue was then re-stored at $-80^\circ$C until ARG was performed.

For D1 receptor binding, slides were removed from $-80^\circ$C freezer and warmed up to room temperature. The slides were circled with nail polish and once dried, they were pre-washed in Tris-HCl buffer (50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 2 mM CaCl$_2$ and 1 mM MgCl$_2$, all from Sigma) for 15 minutes at 20$^\circ$C and pH = 7.4. After the pre-incubation, the slides were laid out to dry in the incubation trays with wet paper towel strips to keep the tissue moist. Incubation solution was made from the pre-incubation buffer with 2 nM $[^3]$H SCH 23390 (Perkin Elmer, Canada; Specific Activity = 62.436 Ci/mmol) and 20 nM ritanserin (Sigma) to block serotonin receptors. Non-specific binding was determined in adjacent tissue slides with an addition of 10μM (+)-butaclamol (Sigma) to the same incubation solution as above. The slides were incubated for 45 minutes with respective total or non-specific binding incubation buffers. After the incubation, the slides were washed twice for 3 minutes in post-wash buffer at 4$^\circ$C and followed by a brief dip in cold, distilled, deionised water, also at 4$^\circ$C. The slides were then laid out on the bench to dry overnight. The following day, the slides were post-fixed in paraformaldehyde powder under vacuum in
a desiccator for at least 24 hours (Liberatore’99). Then the slides were apposed to tritium-sensitive phosphor screens (Fuji Medical System Inc., Stamford, CT) with $[^3]H$ microscales (Amersham, UK) in standard film cassettes. In order to erase any prior residual underlying images on the screen, the screens had been erased before exposure by placement on a light-box for a minimum of 10 minutes. On the fifth day, the screens were removed from the cassettes and scanned in the Cyclone phosphor imager (Perkin-Elmer) at a resolution of 600 dpi.

2.5.3 Data Analysis

Data analysis of ARG followed the method described in Strome et al. (Strome et al., 2005). The binding data was analyzed using the Optiquant v.4.0 software (Packard), which measures the light intensity in digital light unit (DLU). Two small circular regions of interest (ROI area = 7.4 mm$^2$ for anterior, 10.6 mm$^2$ for medial sections) were placed on the striatum (Figure 12). Striatal total binding was measured over 6 sections per animal, on two different levels of anterior and medial sections. Similarly, non-specific binding was measured and subtracted from the total binding on two intermediate slices at each level. Standard curves were derived from commercial $[^3]H$ microscales that were calibrated for 20μM tissue sections (Figure 11). The standard values (nCi/mg) were divided by DLU measured from each microscale to get a slope of linear regression. The optical density data were converted to nCi/mg tissue using a standard curve derived from the $[^3]H$ microscales.
Figure 12: Placement of ROI for standard curve and brain section. The left side of the slide (1, 2, and 3) consists of lesioned hemisphere and the right side (4, 5, and 6) consists of healthy hemisphere. Background DLU was obtained from placing an ROI (7) in unexposed area of the screen.

Figure 13: Anterior and Medial sections and placement of ROI. T1 and T2 relate to templates for the anterior section and T3 and T4 for the medial section. The ROIs are placed on the dorsal striatum where there is hottest binding (darkest area). Note the size difference in ROI between anterior and medial slides.
2.6 Statistical Analysis

A two-way 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 Bonferroni post-test. All statistical analyses were performed using the software program GraphPad Prism v.3.0 for Windows (San Diego, CA).
Chapter 3: Results

All ECS treated animals survived the treatment procedure and entire post-treatment period. All animals received ketamine anesthesia and recovered well and there were no signs of morbidity throughout the experiment.

3.1 Evaluation of 6-OHDA Lesion

All animals that received 6-OHDA lesion were evaluated through non-pharmacological behaviour test. The tail flick test was used to evaluate the severity of lesion by observing the rotational behaviour of the rats. The animals were scored from 0 – 3 based on the number of turns they made during 15 seconds; the scores 2-3 indicated good lesion, 1 indicated partial lesion and 0 meant the rat was not lesioned properly.

All animals that showed good lesion were randomly selected into sham or ECS treatment groups. There were no sign of morbidity throughout the experiment and only two animals (one from each group) were lost due to anesthesia. The time duration of seizure ranged from 10 seconds up to 25 seconds, and most animals showed some tonic hind limb extension indicating a generalized seizure. Refer to Table 1 for the status of each animal.
<table>
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<th># days with Tonic Hind Limb Extension (THLE)</th>
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Table 1: The status of ECS-Treatment group. The table shows number of days that the animal seized more than 13 seconds (minimum), number of tonic hind limb extension and the total current applied throughout the ECS treatment.
3.2 Behavioral Result

3.2.1 Behavior prior to Treatment

Prior to any interventions, all animals were evaluated with the tail flick test and the tapered ledged beam-walking test to determine the normal parameters of animal performance. To evaluate the development of motor deficits and assess the variability and reproducibility of both tests, all animals were tested two weeks after the 6-OHDA lesion.

3.2.2 Tail flick test

Prior to lesioning, the animals made none or one spontaneous rotation. Two weeks after the lesion, the animals were tested again and severely unilaterally lesioned animals made spontaneous ipsilateral rotations. Figure 14 shows data for the animals' rotational behavior prior and after the 6-OHDA lesion. A one-way repeated measures ANOVA was performed to assess the effect of unilateral lesion of 6-OHDA on rotational behavior. There was a significant effect of lesion on the percentage of rotation in both sham- ($F_{2,48} = 154.8$, $p<0.0001$) and ECS-treated groups ($F_{2,50} = 87.36$, $p<0.0001$). Post-hoc comparisons using the Tukey's multiple comparison test indicated that the average number of turns increased significantly after unilateral 6-OHDA lesion ($p<0.001$) compared to the pre-lesion percentage turns.
Figure 14: Tail flick test score before and after 6-OHDA lesioning for Sham and ECS treatment groups.

Bars represent mean % turns (±SEM), n = 50 animals per group. SEM = standard error of the mean. Animals in both groups made significantly higher percentage of turns post lesion (Sham group: Mean (M) = 84.19%, Standard Deviation (SD) = 22.92; ECS group: M = 88.22%, SD = 16.06) than pre-lesion (Sham: M = 7.12%, SD = 10.91; ECS: M = 3.78%, SD = 5.72). After lesion, there is a significant increase of the % turns for both sham and ECS treatment group.

*** Significantly different from pre-lesion (p<0.001).
3.2.3 Tapered ledged beam-walking test

Prior to lesioning, the animals in both group made very few errors and those only occurred on the narrow section of the beam. Severely unilaterally lesioned animals made mistakes mainly in the narrow section of the beam, with their hind limb contralateral to the lesion (only these data are shown). A one-way repeated measures ANOVA was performed to assess the effect of lesion on the percentage of errors/steps in the narrow section of the beam for each group. There was a significant effect of lesion in both sham- \((F_{2,50} = 32.29, p<0.0001)\) and ECS-treated groups \((F_{2,50} = 23.70, p<0.0001)\). Figure 15 shows beam test score for the hind limb contralateral to the lesion of two treatment groups pre- and post-lesion. Post-hoc comparisons using the Tukey’s multiple comparison test indicated that the percentage errors/steps increased significantly after unilateral 6-OHDA lesion \((p<0.001)\) compared to the pre-lesion percentage errors/steps.
Figure 15: Left hind limb mean (±SEM) percentage of errors/steps on the narrow section before and after 6-OHDA lesioning, n = 50 animals per group.

SEM = standard error of the mean. The amount of increase of mean percentage errors/steps for both groups is similar with pre lesion (Sham: M = 10.52%, SD = 19.63; ECS: M = 13.42%, SD = 17.64) and post lesion (Sham: M = 45.99%, SD = 22.79; ECS: M = 51.59%, SD = 25.18). There is a significant effect with lesion in both groups, with increased % errors/steps following the lesion.

*** Significantly different from pre-lesion (p<0.001).
3.3 Behavioral effect of ECS treatment in a Unilateral 6-OHDA Rat Model of PD

In order to assess the behavioral effects of ECS treatment over time in this model, all animals were lesioned along the right MFB with 6-OHDA as described before (Refer to Chapter 2). Two weeks after unilateral 6-OHDA, animals either received ECS treatment (n = 25) or sham-treatment (n = 26) for ten consecutive days. Behavioural testing was done prior to lesion, 2 weeks after lesion (before treatment), and three different time points after treatment at 2, 14 and 28 days, using the tail flick test and the beam test. The data for 2 and 14 days post-treatment were combined between the groups to constitute one data for the purposes of increasing the statistical power.

3.3.1 Tail flick test

A two-way repeated measures (mixed model) ANOVA was performed to assess the treatment x time (post-lesion, 2 days, 14 days and 28 days after treatment) interaction on the percentage of turns that animal makes. There was a significant treatment x time interaction at all time points; at 2 days post-treatment ($F_{1,49} = 8.57$, $p<0.005$), 14 days post-treatment ($F_{2,60} = 5.11$, $p<0.01$) and at 28 days post-treatment ($F_{3,36} = 9.07$, $p<0.001$).

Bonferroni’s posttest indicated that for animals in ECS-treated groups had significant decrease in percentage turns 2 days post-treatment ($p<0.001$) compared to pre-treatment. Sham treated groups showed no significant effect of treatment (Figure 16). At 14 days post-treatment, the effect of ECS treatment on the percentage turns was extremely significant ($p<0.001$) (Figure 17). However, there was no observable significant effect of ECS treatment
at 28 days post-treatment when compared to pre-treatment score (Figure 18). For the sham-treated group, there was no significant effect of treatment on percentage turns at all three time points.
Figure 16: Tail Flick data for ECS- and sham-treated rats before and 2 days after repeated treatment.

ECS-treated rats show lower scores after the treatment compared to sham-treated controls. There is a significant treatment x time interaction \( (F_{1,49} = 8.57, p<0.005) \) with ECS-treated rats showing lower \% turns compared to sham-treated controls. The animals in ECS-treatment group show significant decrease \% turns at 2 days post-treatment \( (**p<0.001) \) compared to pre-treatment score. Bars represent mean \% turns (±SEM), \( n = 25 \) animals per group. SEM = standard error of the mean.
Figure 17: Tail Flick data for ECS- and sham-treated rats before, 2 days after and 14 days after repeated treatment.

There is a significant treatment x time interaction before and at 14 days after treatment ($F_{2,60} = 5.11$, $p<0.01$). Both ECS- and sham-treated rats show a decreased % turns compared to pre-treatment score. However, only ECS-treated rats show a significant effect of treatment at 2 days post- (***$p<0.001$) and at 14 days post treatment (***$p<0.001$). Bars represent mean % turns (±SEM), n = 16 animals in sham-treated control and n = 15 animals in ECS-treated group. SEM = standard error of the mean.
Figure 18: Tail Flick data for ECS- and sham-treated rats before, 2 days, 14 days and 28 days after repeated treatment.

There is a significant treatment x time interaction with ECS treated rats showing lower % turns at 2 days post-treatment (**p<0.01) and 14 days post-treatment (***p<0.001) compared to sham-treated control. However, there is no significant effect of treatment at 28 days after the treatment. Bars represent mean % turns (±SEM), n = 8 animals in sham-treated group and n = 7 animals in ECS-treated group. SEM = standard error of the mean.
3.3.2 Tapered ledged beam-walking test

As previously mentioned, severely unilaterally lesioned animals made most mistakes in the narrow section, so only these data are shown.

Two animals that showed no motor deficits were excluded from this analysis. A two-way repeated measures (mixed model) ANOVA was performed to assess treatment x time (pre-, 2, 14, and 28 days post-treatment) interaction on the percentage of errors/steps on the contralateral hind limb to the lesion. There was a significant treatment x time interaction at 2 days post-treatment ($F_{1,47} = 7.77, p<0.01$) and on 28 days post-treatment ($F_{3,39} = 3.67, p<0.02$), but not quite significant effect of treatment for 14 days post-treatment group ($F_{2,62} = 2.43, p=0.09$).

Bonferroni's posttest was performed to assess the effect of ECS treatment before and after repeated ECS or sham treatment. The animals in ECS-treated group showed a significant decrease in percentage of errors/steps at 2 days post-treatment ($p<0.01$) compared to pre-treatment. There was no effect of repeated sham-treatment on the beam test scores (Figure 19). There was a significant decrease in percentage of errors/steps ($p<0.05$) at 14 days post-treatment for ECS-treated group (Figure 20). At 28 days post-treatment, there was a significant treatment x time interaction ($p<0.05$) in ECS-treated group but no significant effect in sham-treated controls (Figure 21).

The effect of ECS treatment on the ipsilateral hind limb was also assessed. A two-way repeated measures (mixed model) ANOVA was performed to assess treatment x time (pre-,
2, 14, and 28 days post-treatment) interaction on the percentage of errors/steps on the ipsilateral hind limb to the lesion. There was no significant effect of ECS treatment detected at all time points post-treatment.

We also performed Bonferroni’s posttest to assess the effect of ECS or sham treatment on the average % errors of ipsilateral hind limb. The animals in ECS-treated group showed a significant decrease in percentage of errors/steps at 2 days ($p<0.05$) and at 14 days post-treatment ($p<0.05$) compared to pre-treatment. However, no significant effect was detected in the 28 days post-treatment group. All sham-treated controls show no significant effect on the beam test score.
Figure 19: Beam data for contralateral and ipsilateral hind limb on the narrow section of the beam in ECS- and sham-treated rats before and after repeated treatment (2 days).

For the contralateral hind limb to the lesion, there is a significant treatment x time interaction ($F_{1,47} = 7.77, p<0.01$) with ECS-treated rats showing lower % errors/steps compared to sham-treated controls. The animals in ECS-treatment group show very significant decrease in % errors/steps at 2 days post-treatment (**$p<0.01$) compared to pre-treatment score. The animals in ECS-treated group also show significant decrease in % errors/steps for the ipsilateral hind limb (*$p<0.05$). Bars represent mean % errors/steps ($\pm$SEM), $n = 25$ animals per group. SEM = standard error of the mean.
Figure 20: Beam data for contralateral and ipsilateral hind limb on the narrow section of the beam in ECS- and sham-treated rats before and after repeated treatment (14 days).

The interaction of treatment x time for contralateral hind limb is considered not quite significant ($F_{2,62} = 2.43$, $p=0.09$). Compared to the sham-treated controls, the ECS-treated rats show lower beam test score after treatment. There was a significant effect of treatment at 14 days post-treatment ($p<0.05$) on the contralateral hind limb. Also, the animals in ECS-treated group also show significant decrease in % errors/ steps for the ipsilateral hind limb ($p<0.05$). Bars represent mean % errors/steps ($\pm$SEM), n = 16 animals in sham-treated control and n = 15 animals in ECS-treated group. SEM = standard error of the mean.
There is a significant treatment x time interaction ($F_{3,39} = 3.67, p<0.02$) with ECS treated rats showing lower % errors/steps after treatment compared to sham-treated rats. There is a significant treatment x time interaction at 14 days post-treatment ($**p<0.01$) and at 28 days post-treatment ($*p<0.05$). However, no significant effect of treatment is detected in sham-treated controls. For the ipsilateral hind limb, there is no effect of treatment on the beam test score. Bars represent mean % errors/steps (±SEM), n = 8 animals in sham-treated group and n = 7 animals in ECS-treated group. SEM = standard error of the mean.
3.4 Autoradiography Analysis

Binding study of D1 receptors in the dorsal striatum was performed using $[^3\text{H}]$SCH 23390 and the specific binding of this radiotracer was homogenous throughout the striatum. Figure 22 shows the images of $[^3\text{H}]$SCH 23390 binding obtained by in vitro ARG.

There were no significant effects of treatment, or significant interaction between the treatment and hemisphere, indicating that ECS treatment had no effect on D1 receptor binding in the dorsal striatum. Figures 23 - 25 show the effect of ECS or sham treatment on D1 receptor binding in lesioned and non-lesioned hemisphere for different time points.
Figure 22: Image of $[^3\text{H}]\text{SCH 23390}$ binding obtained by in vitro autoradiography in ECS treated rats.

Slides 1 – 3 show the anterior section and slides 4 – 6 show the medial section of the striatum. Total binding was measured by incubating slides in a buffer containing 2nM $[^3\text{H}]\text{SCH 23390}$ for 45 minutes (Slides 1,2,4,5; darker areas); non-specific binding was measured by addition of 10μM (+)-butaclamol (slides 3 and 6).
Figure 23: [³H]SCH 23390 binding in the dorsal striatum at 2 days post-treatment.

There was no significant effect of ECS treatment ($F_{1,32} = 0.78, p = 0.38$). Data are shown as mean binding ±SEM, n = 9 animals per group. SEM = standard error of the mean.

Sham Group: lesioned (M = 8.68, SD = 0.99), non-lesioned (M = 8.89, SD = 1.18)

ECS Group: lesioned (M = 9.06, SD = 2.01), non-lesioned (M = 8.40, SD = 1.56)
Figure 24: $[^3H]$SCH 23390 binding in the dorsal striatum at 14 days post-treatment.

There was no significant effect of ECS treatment ($F_{1,32} = 0.03, p=0.86$). Data are shown as mean binding ±SEM, n = 9 animals per group. SEM = standard error of the mean.

Sham Group: lesioned (M = 7.97, SD = 2.83), non-lesioned (M = 8.33, SD = 2.74)

ECS Group: lesioned (M = 8.61, SD = 2.89), non-lesioned (M = 8.65, SD = 2.57)
Figure 25: $[^3]$H]SCH 23390 binding in the dorsal striatum at 28 days post-treatment.

There was no significant effect of ECS treatment ($F_{1,26} = 0.04$, $p=0.85$). Data are shown as mean binding ±SEM, $n = 7 - 8$ animals per group. SEM = standard error of the mean.

Sham Group: lesioned (M = 6.76, SD = 1.55), non-lesioned (M = 6.94, SD = 1.41)

ECS Group: lesioned (M = 7.42, SD = 1.72), non-lesioned (M = 7.37, SD = 1.83)
Chapter 4: Discussion

The purpose of this study was to investigate the effects of repeated ECS treatments in 6-OHDA lesioned rats. Using two different types of non-pharmacological behavioural tests and post mortem ARG, the persistence of motor symptoms improvements and changes in DA receptors was examined. Our findings showed significant improvements in motor behaviour with repeated ECS treatments, which lasted up to 2 weeks; by 4 weeks after the last ECS treatment, there was loss of improvement in some of our behavioural tests. Based on the existing literature, we hypothesized there would be changes in dopamine function to parallel improvements in motor function, for example up-regulation of DA D1 receptors in the striatum, as reported in earlier studies. However, we did not reproduce the earlier data and found no significant changes in D1 binding between ECS treated and sham-treated animal for all three time points.

ECT treatment appears to be a suitable adjunct treatment option for PD patients with co-morbid depression due to its antidepressant effects, as well as its immediate and long-lasting anti-parkinsonian effects (Andersen et al., 1987; Zervas and Fink, 1991). Several reports show that repeated ECT treatments in PD patients lead to a dramatic improvement in motor symptoms that last for weeks to months (Fall et al., 1995b; Pridmore and Pollard, 1996). However, very few studies have investigated the central mechanisms behind such phenomena, and studies with 6-OHDA lesioned rats are especially scarce. Most studies using animal models have looked at drug-induced behaviour and showed significant increase in DA mediated behaviour after repeated ECS treatments (Wielosz, 1981; Green et al., 1983; Green
et al., 1977; Anastasia et al., 2007). Therefore, we decided to investigate the mechanism of ECS using non-pharmacological behaviour tests in 6-OHDA lesioned rats.

This thesis was based on pilot studies that investigated the effect of repeated ECS treatments in the striatal regions of unilateral 6-OHDA lesioned male rats. Significant improvements in gross motor function as well as enhanced expression of striatal D1 and D3 receptor binding were observed (Strome et al., 2007). However, these preliminary studies only looked at one time point, 48 hours following treatment, thus the persistence of these effects is unknown. We therefore repeated the existing pilot studies and added two other time points, 14 and 28 days post ECS. We used the same methods that were used in the pilot studies with only one difference: we used female rats instead of male due to their smaller body size, which made it easier to generate good seizures with our old ECS machine.

4.1 Behavioural Data

Drug-induced motor behaviour using apomorphine or amphetamine is frequently used to evaluate the efficacy of potential anti-parkinsonian treatments (Green et al., 1977; Anastasia et al., 2007). However, these pharmacological agents have been shown to induce their own intrinsic responses, such as alteration of DA receptors, which make the longitudinal (i.e. repeated exposure) studies more difficult to interpret. This thesis used non-pharmacological tests that rely on spontaneous motor function of normal rodent behaviour to assess the pre- and post-treatment behaviour. The tail flick test allowed us to test the rotational behaviour and the beam test allowed us to study the gross motor function.
4.1.1 The Tail Flick Test

Motor asymmetries in unilateral 6-OHDA rats are reflected in the development of directional bias in locomotion. Animals typically display spontaneous ipsilateral rotation towards the lesion side (Costall et al., 1976; Dunnett and Iversen, 1982), which is related to the degree of striatal activity. This rotational behaviour is interpreted as being opposite in direction to the side of brain with the highest dopaminergic activation (Lane et al., 2006). Therefore, rotation to the right, as seen in right unilateral 6-OHDA lesioned rats, indicates an action of the intact left striatum and lack of activity on the denervated right striatum. The tail flick test is also often used to measure the severity of lesion. We, however, found it to be a sensitive index of therapeutic effect.

Surprisingly, no reports have been made on the use of the tail flick test to study anti-parkinsonian effects. Instead, most studies looked at the rotational behaviour of 6-OHDA lesioned animals through drug-induced tests (Green et al., 1977; Anastasia et al., 2007). In order to eliminate potential intrinsic response due to pharmacological agents, we used non-pharmacological tests to assess behavioural responses. In this study, we found that normal rats did not spontaneously rotate, but that upon 6-OHDA lesion, all rats developed motor asymmetry and rotated to the side of lesion. ECS-treated animals made significantly less turns compared to sham-treated groups; this was observed until at least two weeks after the last ECS treatment. However, by four weeks post-treatment, there was a loss of improvement in the ECS-treated animals, and they made more average spontaneous rotations, suggesting a return to baseline.
4.1.2 The Tapered Ledged Beam Walking Test

All 6-OHDA lesioned rats were assessed pre- and post-treatment (2 days, 14 days and 28 days) using a tapered ledged-beam walking test, which was modified by the addition of a transparent ledge spanning the beam’s length to prevent compensational behaviour. The beam test is a widely used behaviour assessment and is a valid test of DA-dependent motor function (Cepeda et al., 2007a; Strome et al., 2007). After the right unilateral 6-OHDA lesion, most animals displayed their motor impairment by using the ledge as support for the contralateral hind limb to the lesioned striatum, especially in the narrow section.

The relationship between performance on the task and the integrity of the striatal DA system has been studied previously using unilaterally lesioned rats with RPE cells implants in the lab (Cepeda et al., 2007a). There was a significant improvement on the beam test with anti-parkinsonian intervention, portrayed by a decreased number of foot-faults (errors) on the contralateral hind limb. Similarly, our pilot studies show significant improvement in the beam test scores after repeated ECS treatment (Strome et al., 2007). In this thesis, we expected to detect similar improvements in gross motor function and persistence of such improvements with repeated ECS treatments.

There was a significant improvement in the beam test score in ECS-treated animals, which lasted up to 4 weeks post treatment. ECS-treated rats displayed a strong treatment by time interaction, and there was up to 15% reduction in error at 2 and 14 days post treatment compared to the pre-ECS treatment time point. It is important to note that the average % errors were higher at 28 days (39.88%) than at 14 days post-treatment (34.34%), suggesting a
possible return to baseline. In addition, ECS-treated rats made significantly less average %
errors on the ipsilateral hind limb (right side) compared to sham-treated controls. This
observation lasted at least two weeks post-treatment, but no significant motor improvement
was observed by four weeks post-treatment.

4.1.3 Behavioural Analysis

Our data on the effects of repeated ECS treatment in 6-OHDA lesioned animals are generally
consistent with the literature on the positive effects of ECS on motor impairment. First of all,
our tail flick data are in agreement with previous studies that have examined rotational
behaviour after ECS treatment in normal and 6-OHDA lesioned rats. These reports showed
significant increase in drug-induced (amphetamine and/or apomorphine) motor
improvements in animals with ECS treatment (Wielosz, 1981; Green et al., 1977; Green et al.,
1983; Zarrindast et al., 2004; Anastasia et al., 2007). We also detected a significant
improvement in the beam test on both contralateral and ipsilateral hind limbs after repeated
ECS treatment, which is consistent with the pilot studies (Strome et al., 2007). Based on the
behavioural tests, our data show that repeated ECS treatment in unilateral 6-OHDA lesioned
rats has a beneficial effect on the motor impairment lasting at least two weeks, with mixed
improvement by four weeks post-treatment.

The beneficial effects of repeated ECS treatment on the motor symptoms may be due to
enhanced neurotrophic factors, such as BDNF, GDNF and FGF-2, in the nigrostriatal
pathway (Strome et al., 2007; Anastasia et al., 2007). Most animal studies on the effects of
ECT have been mainly focused on the depression model, where there are consistent findings
on the alteration brain-derived neurotrophic factor (BDNF) levels. In these studies, depressive behaviours were correlated with a decreased level of BDNF in the hippocampus and the striatum, whereas anti-depressant treatment enhanced the expression of BDNF (Fumagalli et al., 2007; Monteggia, 2007). In PD, BDNF was found to co-localize with DA neurons in the substantia nigra, where dopaminergic cell bodies are located. Such studies suggest that BDNF has a potent neuroprotective role on survival and morphology of nigral dopaminergic neurons, as its loss contributes to death of these cells in PD (Tooyama et al., 1993; Mogi et al., 1999; Chauhan et al., 2001). Repeated ECS treatment in normal animals also increased the concentration of mRNA and protein for BDNF in the hippocampus, cortical regions and the striatum (Nibuya et al., 1995; Angelucci et al., 2002; Altar et al., 2003; Jacobsen and Mork, 2004). In addition, pilot studies showed an increased level of BDNF protein in the striatum with repeated ECS treatment. These preliminary data suggest that ECS treatment may indirectly improve motor function by enhancing neurotrophic factor expression and promoting an increased BDNF level in the striatum. Furthermore, increased BDNF activity may protect nigral dopaminergic neurons, thereby improving motor function.

As mentioned previously, significant motor improvement is observed in ECS-treated animals, which lasted at least two weeks post-treatment. One possible explanation for such phenomena may be the role of ECS in enhancing brain DA function. In many behavioural studies on rodents, repeated ECS treatment is associated with an increase in DA-mediated behaviour (Bergstrom and Kellar, 1979). For example, repeated administration of ECS in normal rats lead to significant improvement in apomorphine and amphetamine-induced motor behaviour, such as rotation (Wielosz, 1981; Zarrindast et al., 2004; Green et al., 1977).
Similar effects are also detected in DA-depleted animals, where repeated ECS treatment shows improvement in rotation and hind limb functions (Green et al., 1983; Anastasia et al., 2007; Strome et al., 2007). These improvements in DA-mediated behaviour may depend on the neurochemical alterations due to ECS treatment. For example, studies on the effects of repeated ECS treatment on DA receptors consistently show up-regulation of D1 receptors in the normal striatum after a course of ECS (Fochtman et al., 1989; Nowak and Zak, 1989; Barkai et al., 1990; Strome et al., 2007), while D2 receptors remain unchanged (Sershen et al., 1991; Martin et al., 1995; Strome et al., 2007). In addition, the pilot studies show increased binding to both D1 and D3 receptors in the striatum but no change in binding to D2 receptors (Strome et al., 2007). Based on these literature and small pilot studies on the effects of ECS on DA receptors, we decided to investigate the relationship between nigrostriatal dopaminergic function and the motor behaviour. We specifically chose to investigate the D1 receptors, as their up-regulation following repeated ECS treatment, which may potentially play a significant role in motor output, is frequently observed in numerous literatures.

4.2 In vitro Autoradiography

A number of studies suggest that ECS treatment does not affect D2 receptors but increases D1 receptor function (Strome et al., 2007; Nowak and Zak, 1989; Barkai et al., 1990; Sershen et al., 1991; Martin et al., 1995). In particular, it has been hypothesised that changes in motor behaviour may be driven by DA D1 function by increasing the activity levels of D1 receptors. Based on the previous literature on the effects of repeated ECS on DA receptors in the normal rat brain, we expected to find increased D1 receptor binding in the dorsal striatum.
(Nowak and Zak, 1989; Barkai et al., 1990). Also, in the pilot studies, repeated ECS treatment in unilateral 6-OHDA lesioned rats showed a significant increase in binding of D1 receptors in lesioned striatum (Strome et al., 2007).

However, our findings are inconsistent with the previous studies on the effect of repeated ECS treatment on DA receptors; we observed no change in D1 receptor binding following repeated ECS treatment. There were also no significant effects of treatment by time on D1 receptor binding in both lesioned and non-lesioned striatum. In short, the findings of our study do not support the hypothesis that the D1 receptor system may be responsible for the prolonged effect in motor function.

The role of gender in responsiveness to ECS treatment may explain the discrepancy between our data and the literature. It is widely understood that there are gender discrepancies in both the PD and the depression model where there is a difference in monoaminergic system (Cyr et al., 2002; Diamond et al., 1990; Tamas et al., 2006). Females are believed to have a more resilient system, such that they are diagnosed less frequently with PD but more frequently with depression. However, there are very few studies that investigate the role of gender in ECT treatment. In particular, most animal studies of PD have used male rats but, due to technical issues, we used female rats to ensure effective induction of seizure in all rats. It is therefore possible that there is less significant effect of repeated ECS treatment in D1 receptor binding due to the gender difference. Thus, it will be crucial for future studies to investigate the role of gender in both behavioural improvements and DA receptor binding after repeated ECS treatment.
Another explanation on the discrepancy in D1 receptor binding may be due to the ability or inability to generate seizure. In depression, it is widely understood that the therapeutic effects of ECT are due to seizures (Ishihara and Sasa, 1999; Cronholm and Otosson, 1996). It is unknown, however, whether the therapeutic benefit of ECT in PD is due to seizures or to the electrical stimulation. In the pilot studies, all animals that underwent ECS treatment showed good tonic hind limb extension (THLE), which is indicative of full-generalized body seizure (Strome et al., 2007). However, in our studies, the animals in the 14 days and 28 days groups showed a moderate level of seizure but with a lesser number of THLE. It may also be important to consider whether a full-generalized body seizure is necessary to detect changes in DA receptors in the striatum of parkinsonian animals after repeated ECS treatment.

4.3 Limitations of the studies

The 6-OHDA rodent of model PD was first developed in the 1960s (Ungerstedt, 1968; Schallert et al., 1978; Lee et al., 1996) and has been extensively studied. Rodent PD models with 6-OHDA lesions, as well as monkey PD models created with MPTP, have been recognized as very reliable PD models and are widely used for neuro-chemical and pharmacological studies. This model of PD also complements studies in primates with high clinical validity (Cenci et al., 2002). However, we must note that some features of the 6-OHDA-lesion rat model of PD are not completely representative of the clinical situation. For example, in this model, there is an extreme loss of striatal DA neurons in one hemisphere of the brain and, subsequently, severe motor deficits on one side of the body. In reality, PD
patients suffer DA depletion and motor symptoms bilaterally and their nigrostriatal DA neurons are only half lost. In future studies, it would be ideal to look at different lesion techniques (unilateral 6-OHDA versus partial lesion model) to find a more appropriate model.

4.4 Future Directions

Given the ability to perform this study again, there are several previously unexplored factors, which I would wish to consider. These factors, which will be outlined in further detail below, would contribute to our understanding of the mechanisms of action of ECS in the parkinsonian animal model.

In this study, we used a fairly small number of animals in the 28 days post-treatment set, with only seven or eight rats per group. In the future, I would use a much larger sample size to increase the validity of the results. Furthermore, conducting more experiments with a larger n enable us to detect with greater certainty the persistence of ECS treatment.

Gender may be another factor that caused the variation between the pilot study and our work in ARG experiments. For example, male animals are more susceptible to 6-OHDA than females; female rats have significantly less dopaminergic cell loss (Tamas et al., 2006). Most literature studying the effectiveness of ECS treatments, including the pilot study, has predominantly used male rats. Therefore, it is possible that there may be a difference in neurochemistry of dopaminergic system between males and females; our findings of less significant D1 receptor binding may be due to this gender difference. In future studies, I
would extend the original experiments conducted on male rats, looking at the persistence of behavioural functions and in vitro ARG experiments at three different time points.

Since there are conflicting data with respect to the D1 receptor bindings after repeated ECS treatment, I would also investigate the role that the D1 receptors play in the improvements in motor function. A similar study was performed to see the relationship between chronic L-DOPA treatment and DA receptors (Van Kampen and Stoessl, 2000; Van Kampen and Stoessl, 2003); their findings indicate that the dramatic improvements in behaviour after L-DOPA treatments depend on the D1 and D3 receptors. In future studies, I would knock down the expression of D1 receptor genes using antisense oligodeoxynucleotides to study its role in the motor improvements after ECS treatment. These findings will provide further identification of the DA receptors’ role in the positive effect that ECT plays on the motor symptoms of PD.

The pilot studies showed that, after repeated ECS treatment, there is an increase in both D1 and D3 receptor bindings in the dorsal striatum. Chronic treatment with L-DOPA in 6-OHDA rats led to behavioural improvements as well as moderate level of increase in D3 receptor expression in the lesioned dorsal striatum (Bordet et al., 1997; Van Kampen and Stoessl, 2003). It has been reported that co-expression of D1 and D3 receptors have both opposite and synergistic effects on the nigrostriatal pathway (Strome et al., 2007). High levels of expression of D3 receptors can lead to over-activity of the direct pathway whereas moderate levels of expression (compared to D1 receptors) may contribute to synergistic interaction between D1 and D3 receptors and enhance the activity of the direct pathway.
(Bordet et al., 1997; Bordet et al., 2000). In future studies, I would investigate the role of D3 receptors in motor improvements after repeated ECS treatment. Using brain slices from female rats, I would assess the postsynaptic effects of ECS through in vitro ARG using ligands specific for D3 receptors ([3H]7-OH-DPAT). The findings from these data will allow further understanding of DA receptors, which may be able to explain the improvements in motor function.

The preliminary studies on the effect of repeated ECS treatment on the neurotrophic factors also showed changes in BDNF expression (Strome et al., 2007). I would extend these studies to include female rats in order to explore if gender plays a role in the expression of BDNF. I would also investigate the involvement of BDNF in motor improvements to repeated ECS treatments. To do so, I would infuse a soluble antibody (IgG-TrkB) against BDNF receptor into the rats’ striatum during repeated treatments with ECS, in order to observe changes in both motor behaviour and the expression of DA receptors.

In depression, it is widely understood that the therapeutic effects of ECT are due to seizures. However, it is unknown whether it is electrical stimulation or seizure induction, which plays a role in the positive effects of ECS on the DA system. It is observed that the therapeutic benefit of ECT in PD occurs much earlier than the antidepressant effect. Since, it is observed that the therapeutic benefit of ECT in PD occurs much earlier than the antidepressant effect, I would investigate whether motor improvements could be observed using sub-seizure stimulations. For this I would use two groups of animals, one receiving sub-seizure electrical
stimulation and/with the other receiving seizure induction. Their subsequent motor behaviour and any changes in the DA system could thus be assessed.

4.5 Significance of the Findings

This study examined the mechanisms of action of applying electrical stimulation to treat psychiatric and neurological disorders. It has been observed that, after chronic use of the available pharmacological treatments, many PD patients develop debilitating side effects and that the drugs lose their effectiveness. ECT is considered a safe, effective and widely available treatment option for major depressive disorder. Furthermore, the clinical evidences show that ECT can provide immediate and long-lasting improvements in motor symptoms. Thus, ECT may be a particularly useful therapy for PD patients with co-morbid depression. As this study is the first comprehensive longitudinal study of the effects of repeated ECS treatment on the motor improvement, these results can be used as steps to achieve acceptance and clinical application of ECT as an adjunct treatment for PD.

4.6 Conclusion

To the best of our knowledge, this study provides the first comprehensive investigation of the longitudinal effects of repeated ECS treatment in the 6-OHDA lesioned rats. We have shown that repeated ECS treatment improves motor functions, which lasts at least two weeks and mixed improvement by four weeks post-treatment.
ECT is considered an effective treatment for depression and it also appears to have positive effects in PD, improving motor symptoms for several weeks. While the mechanism of action is not completely known, and further research is necessary, this study shows the prolonged effect of ECT on the motor symptoms and its potential use as an adjunct therapy for PD, particularly in PD patients with co-morbid depression.
References


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