

**Effects of Acute and Chronic Dopaminergic
Treatment on Motor and Non-Motor Function in
the Hemi-Parkinsonian Rat**

This thesis is submitted for the degree of Doctor of Philosophy at Cardiff University

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2014

Thesis summary

Parkinson's disease (PD) is commonly treated with L-DOPA. Long-term treatment is associated with the development of motor side effects such as L-DOPA induced dyskinesia (LID), and pathological changes in the striatal circuitry. However, while this circuitry is implicated in both motor and non-motor behaviour, little is known about the effects of long-term L-DOPA treatment on non-motor function. This thesis used a rat model of PD and LID to test the hypothesis that long-term L-DOPA treatment also affects non-motor behaviour.

Pharmacological studies typically utilise albino rats, while pigmented rats are preferred for operant studies. To guide later methodological decisions, **Experiment 1** compared pigmented Lister Hooded and albino Sprague Dawley rats' motor, dyskinetic, or operant response to L-DOPA. As there were no gross strain differences, and Lister Hooded rats are preferred in operant studies, they were used in later experiments measuring both LID and operant behaviour. **Experiment 2** aimed to identify appropriate lesion screening tests. Success of unilateral 6-OHDA lesions is commonly measured using amphetamine-induced rotations. However, amphetamine interferes with goal-directed behaviour. The ability of amphetamine-induced rotations and four non-pharmacological motor tests to accurately identify lesion rats was therefore compared. The non-pharmacological spontaneous rotations and cylinder tests were identified as robust screening tests and used in later experiments.

L-DOPA competes with dietary amino acids for transport across the blood-brain barrier, and chronic L-DOPA treatment sensitises dopamine receptors. It was therefore hypothesised that food restriction and chronic L-DOPA would both decrease the L-DOPA dose required to alleviate motor symptoms in the rat 6-OHDA model. **Chapter 4** describes two dose response curves testing the effect of food restriction and chronic L-DOPA on the motor response to acute L-DOPA in rats with intra-striatal or MFB lesions. Chronic L-DOPA increased the motor response to acute L-DOPA in the MFB, but not intra-striatal, lesion model. Conversely, food restriction increased the motor response to acute L-DOPA in the intra-striatal, but not MFB, lesion model. **Chapter 5** used microdialysis to test the hypothesis that the increased motor response following food restriction was caused by an increased influx of L-DOPA to the striatum. The data did not support the hypothesis but suggested that food restriction affects baseline neurotransmitter levels in the 6-OHDA lesion rat.

Chapter 6 tested the hypothesis that LID onset, by disrupting cortico-striatal synaptic plasticity which is implicated in motor skill learning, impairs acquisition of novel motor skills by measuring rats' performance on the staircase task. While an initial experiment suggested that chronic LID onset impaired lesion rats' acquisition of the task, the phenomenon could not be replicated. **Chapters 7 and 8** further explored the effect of chronic L-DOPA on non-motor function using a lateralized choice reaction time task reliant on the striatal system. Chronic L-DOPA exacerbated a lesion induced accuracy deficit that has been hypothesised to reflect extinction. This deficit was linked to LID onset, rather than L-DOPA exposure *per se*. The data therefore expand on current knowledge by suggesting that the effects of chronic L-DOPA extends beyond inducing motor side effects to also affect non-motor function.

Acknowledgements

First and foremost, a big thank you to my friends, family, and my wonderful boyfriend Sach for your support during both the lab and thesis writing days. You have all been amazing and I cannot thank you enough for your support and encouragement. I promise to now return to the land of the sociable...

In addition to thanking Steve for his input as supervisor I would like to extend a special thank you to the following people who played a particular role in terms of providing scientific help and advice:

- **Mariah Lelos:** a big thank you for all the stimulating scientific discussions, and your valuable input during the thesis writing. You are a true role model and I am extremely grateful for all your help and support.
- **Ludivine Breger:** thank you both for all the interesting dyskinesia discussions and for all of the equally interesting non-work chats. Best of luck with your post-doc studies. Academia is lucky to have you!
- **Sophie King & Jane Heath:** for being great immunohistochemistry teachers as well for your amazing ability to always keep the histology lab running so smoothly. Don't know how you do it!
- **Jane Cooper:** I would not have been able to do the microdialysis without your help! Thank you both for all the help with the HPLC, and for teaching me the ins and outs of microdialysis.
- **Mike, Tracy & the rest of 'the Pod':** for your help and scientific input during the microdialysis study, as well as for helping me settling in and making me feel part of the pod during my Lilly days.
- **Clare Finlay:** a big thank you for your help and advice during the Lilly days. Good luck with the last bit of your thesis - know you will do great!
- **Hanna Lindgren:** thank you for your help during the TAT experiment and the many TAT injections.

Abbreviations

| | |
|---|--|
| 5HIAA. 5-Hydroxyindoleacetic acid | LTD. Long-term depression |
| 6-OHDA. 6-hydroxy-dopamine | LTP. Long-term potentiation |
| aCSF. Artificial cerebrospinal fluid | LRRK2. Leucine-rich repeat kinase 2 |
| Ad Lib. Ad libitum | MAO. Monoamine oxidase |
| AIMs. Abnormal involuntary movements | MDPD+. 1-methyl-4-phenyl-2,3-dihydropyridinium cation |
| BBB. Blood-brain barrier | MEP. Motor evoked potentials |
| BOLD signal. Blood-oxygen-level dependent signal | MFB. Medial forebrain bundle |
| COMT. Catechol-o-methyl transferase | MPP. 1-Methyl-4-phenyl-4-propionoxypiperidine |
| CR. Conditioned response | MPP+. 1-methyl-4-phenylpyridinium ion |
| CS. Conditioned stimulus | MPTP. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine |
| DBS. Deep brain stimulation | MT. Medial terminal nucleus |
| DAT. Dopamine active transporter | NAcc. Nucleus accumbens |
| DOPAC. 3,4-Dihydroxyphenylacetic acid | NMDA. N-Methyl-D-aspartic acid |
| EMG. Electromyography | n.s. Not significant |
| fEP. Field evoked potentials | PD. Parkinson's disease |
| FR. Fixed ratio or food restricted | PBS. Phosphate buffered saline |
| GPe. Globus pallidus external segment | PET. Positron emission tomography |
| GPi. Globus pallidus internal segment | PFA. Paraformaldehyde |
| HFS. High frequency stimulation | PINK1. PTEN-induced putative kinase 1 |
| HVA. Homovanillic acid | PSD. Postsynaptic density |
| i.p. intraperitoneal | ROS. Reactive oxygen species |
| ITI. Inter-trial interval | s.c. Subcutaneous |
| LAT. L-type amino acid transporter | SNc. Substantia nigra pars compacta |
| LCRT. Lateralised choice reaction time task | SNr. Substantia nigra pars reticulate |
| L-DOPA. Levodopa | STN. Subthalamic nucleus |
| LFS. Low frequency stimulation | TBS. Tris buffered saline |
| LID. Levodopa induced dyskinesia | |
| TBZ. Tris buffered saline with azide | |

TH. Tyrosine hydroxylase

TMS. Transmagnetic stimulation

TNS. Tris non saline

UPS. Ubiquitin proteasome system

US. Unconditioned stimulus

VTA. Ventral tegmental area

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1. Introduction

1.1 Overview of the general introduction

Parkinson's disease (PD) is a movement disorder which, on a neurological level, is characterised by a gradual loss of dopaminergic neurons in the substantia nigra pars compacta (SNc) and abnormal alpha synuclein inclusions. Behaviourally, PD is characterised by motor symptoms including tremor, rigidity, akinesia, and postural instability as well as non-motor symptoms such as hyposomnia and constipation. On a cognitive level, PD has been suggested to impair functions such as learning dependent on positive reinforcement and attention processes. This introduction will provide an overview of the history, symptoms, and neuropathology of PD, as well as known risk factors for developing the disease. It will thereafter describe common preclinical models and behavioural tests that are used to study PD and anti-parkinsonian drugs in the preclinical setting. Finally, the introduction will provide an overview of pharmacological treatments available to treat PD symptoms and common side effects of these.

Particular emphasis will be placed on describing levodopa (L-DOPA) which is the gold standard treatment in PD. The introduction will describe the effects of acute L-DOPA on motor and non-motor function. Furthermore, it will describe how long-term treatment gives rise to motor complications in the majority of PD patients. It will thereafter describe how the onset of L-DOPA induced dyskinesia (LID), a common motor complication arising from long-term L-DOPA treatment, is associated with changes to striatal dopamine receptors and cortico-striatal synaptic plasticity.

Whereas many studies have explored the effects of *acute* L-DOPA on both motor and non-motor function, the majority of studies exploring the effects of the *long-term* L-DOPA have focused solely on the motor side effects. However, LID onset is associated with pathological changes in the striatum, which is implicated in both motor function and non-motor function. This suggests that the abnormal changes associated with the onset of LID may not only disrupt motor function, but also affect non-motor functions dependent on the same circuitry. This thesis project therefore tested whether long-term L-DOPA treatment impacts on non-motor behaviour mediated by the striatum using a rat model of PD.

1.2. History and disease overview

The first comprehensive description of PD was provided by James Parkinson in his 1817 monograph “An Essay on the Shaking Palsy”. Parkinson’s description was based on six case studies and stated that patients exhibited:

“Involuntary tremulous motion, with lessened muscular power, in parts not in action and even when supported; with a propensity to bend the trunk forwards, and to pass from a walking to a running pace: the senses and intellects being uninjured” (Parkinson, 1817, p.1)

Parkinson (1817) further described how the first symptom patients recalled was a “slight sense of weakness”. As the disease progressed, patients began experiencing tremor in one limb, usually a hand or an arm, which spread to other muscle groups, usually the contralateral limb, within 12 months. The disease was furthermore associated with a sense of fatigue, a hunched posture, and a spread of the tremor. As the tremor and postural instability worsened, Parkinson (1817) noted that patients also began exhibiting difficulties walking, and an increased risk of falling. In addition, Parkinson (1817) noted an occurrence of non-motor symptoms such as disrupted sleep and hypo-functional bowels movements. Crucial for the scientific understanding of the disease, Parkinson (1817) hypothesised that the characteristic tremors were caused by dysfunction of the central nervous system rather than the muscles.

The motor symptoms described by Parkinson (1817) are similar to what are now considered the cardinal symptoms of PD: involuntary tremor, rigidity, akinesia or bradykinesia, and postural instability (e.g. Jankovic, 2008). In addition, PD also causes a range of non-motor symptoms including REM sleep disruptions, fatigue, excessive daytime sleepiness, hypoactive bowel movements, bladder dysfunction, pain, autonomic dysfunction, dysphagia, and an impaired sense of smell (Siddiqui *et al*, 2002; Chaudhuri *et al*, 2006; Haehner *et al*, 2009; Ziemssen & Reichmann, 2010a,b; Chaudhuri *et al*, 2011). These non-motor symptoms of PD often occur prior to the onset of motor impairments (reviewed in Chaudhuri *et al*, 2006; Hawkes *et al*, 2010) and are perceived by patients as directly decreasing their quality of life (Politis *et al*, 2010).

In addition, PD also affects psychological and cognitive functions. PD is associated with a higher prevalence of anxiety, apathy, depression, and dementia than observed in the general population (Slaughter *et al*, 2001; Chaudhuri *et al*, 2006; van der Hoek *et al*, 2011).

In addition, PD patients show deficits related to frontal lobe dysfunction. These include impaired performance on tasks measuring executive function, such as the Wisconsin Card Sorting Task and reversal learning tasks; which both depend on patients' ability to alter their response strategies according to external feedback (Cooper *et al*, 1991; Swinson *et al*, 2000; Chaudhuri & Schapira, 2009; Kehagia *et al*, 2010). The Wisconsin Card Sorting Task relies on the prefrontal cortex, the parietal lobe, and the striatum (Berman *et al*, 1995; Shafritz *et al*, 2005). Similarly, reversal learning has been shown to rely in the prefrontal cortex, striatum, and orbito-frontal cortex (Kehagia *et al*, 2010). Hence, in addition to the motor symptoms traditionally associated with PD the disease also disrupts higher functions reliant on the frontal-striatal circuitry.

1.3. The basal ganglia

1.3.1. The healthy basal ganglia

PD is presently considered predominantly a basal ganglia disorder. The following section will provide an overview of the basal ganglia circuitry in a healthy brain before describing how the system is affected by PD in section 1.3.2.

The basal ganglia are a group of interconnected nuclei located in the midbrain that share connections with the cortex, thalamus, and brainstem. The main components are the caudate putamen complex (known as the striatum in rodents), the globus pallidus internal (GPi) and external (GPe) segments, the subthalamic nucleus (STN), the SNc, and the substantia nigra pars reticulata (SNr; Alexander & Crutcher, 1990).

The complex interconnections of the basal ganglia are commonly described using the classic direct/indirect pathway model (Figure 1.1). While more complex versions of the model have been developed since the original model, the classic direct/indirect pathway model has remained valuable in describing the basic structure and function of the basal ganglia and is therefore still in use. According to the classic model, the striatum receives excitatory glutamatergic input from the cortex, along with modulatory, dopaminergic input from the SNc. Information is then relayed to the GPi and SNr, the basal ganglia output centres, via either the direct or the indirect pathway.

In the direct pathway, GABAergic neurons containing dynorphin and substance P and expressing D1-type (i.e. D1 and D5) dopamine receptors relay information directly from the striatum to the GPi and SNr (the basal ganglia's output centre) via inhibitory projections. The GPi and SNr are connected to the thalamus via inhibitory GABAergic projections. The

thalamus, in turn, is connected with the motor cortex via excitatory glutamatergic neurons. Activation of the direct pathway results in inhibition of the GPi and SNr. This stops the inhibitory effect these structures otherwise exert on the thalamus, thereby increasing the thalamic input to the motor cortex. Hence, the net effect of direct pathway activation is an increased activity in the motor cortex and increased motor behaviour (Alexander & Crutcher, 1990; Gerfen *et al*, 1990; Smith *et al*, 1998).

In the indirect pathway, inhibitory projections consisting of GABAergic, enkephalin containing neurons expressing D2-type (i.e. D2, D3, and D4) dopamine receptors project from the striatum to the GPe. The GPe, in turn, projects to the STN via further inhibitory, GABAergic projections. Finally, the STN sends an excitatory glutamatergic projection to the GPi and SNr. Activation of the indirect pathway inhibits neuronal activity in the GPe. By decreasing the inhibitory effect that the GPe otherwise exerts on the STN, this ultimately

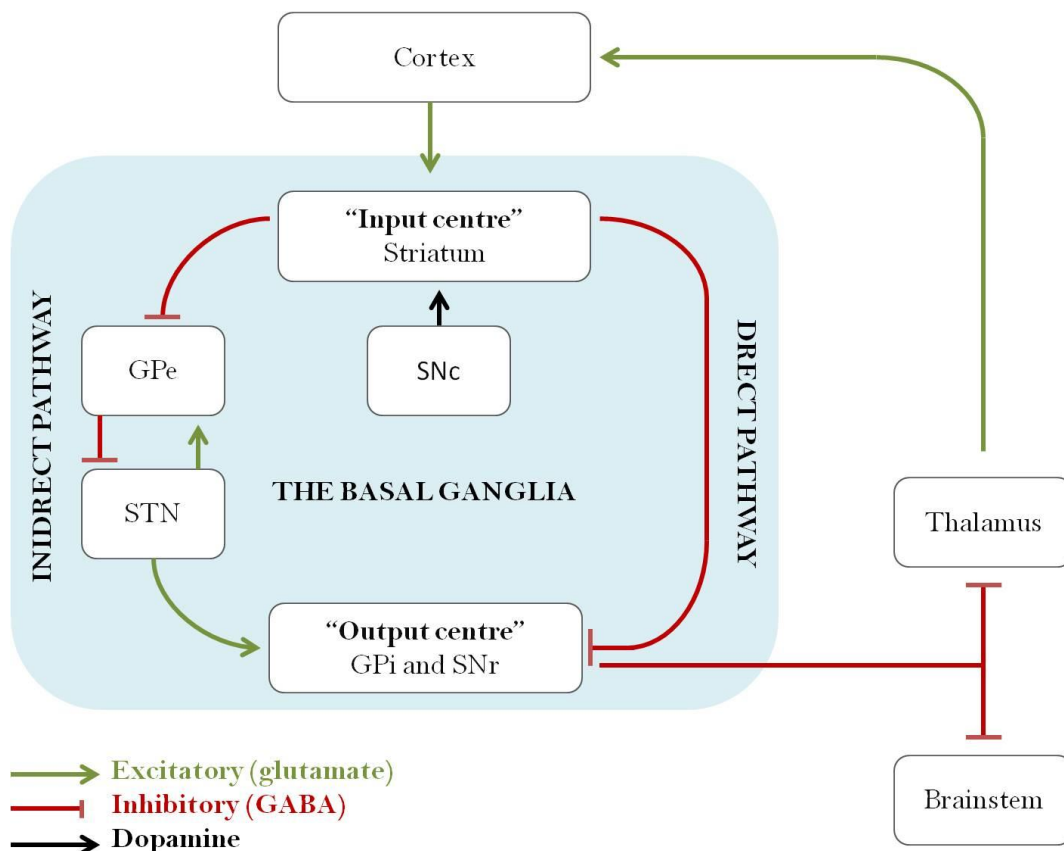


Figure 1.1. A graphical representation of the direct and indirect pathway of the basal ganglia in a healthy brain. GPe=Globus pallidus external segment, GPi=Globus pallidus internal segment, SNc=Substantia nigra pars compacta, SNr=Substantia nigra pars reticulata, STN=Subthalamic nucleus.

causes disinhibition of the STN. When disinhibited, the STN exerts an excitatory glutamatergic influence on the GPi and SNr. Increasing the activity of the GPi and SNr in turn increases the inhibitory effect these structures exert on the thalamus - which in turn decreases the excitatory input the thalamus exerts on the cortex. Ultimately, this results in decreased activity in the motor cortex and reduced motor behaviour. The direct and indirect pathways thus work in parallel, causing excitation and inhibition of motor cortex respectively (Alexander & Crutcher, 1990; Gerfen et al, 1990; Smith et al, 1998).

1.3.2. The basal ganglia in Parkinson's Disease

PD is characterised by a progressive loss of dopaminergic neurons in the SNc (Ehringer & Hornykiewicz, 1960). By the time motor symptoms appear approximately half of the dopaminergic SNc neurons have degenerated, a decrease which correlates with symptom severity and is significantly greater than would be expected in normal ageing (Scatton *et al*, 1983; Fearnley & Lees, 1991). The SNc forms an integral part of the basal ganglia network and the dopamine loss that occurs in PD disrupts the balance that otherwise exists between the direct and indirect pathways. The following section will provide an overview of these pathological changes and their involvement in PD motor symptoms.

Dopamine modulates striatal GABA transmission via D1 and D2 receptor binding. Binding to D1 type receptors excites, whereas binding to D2 type receptors inhibits striatal neurons (Girault *et al*, 1986; Yamamoto & Davy, 1992). The extensive dopamine loss occurring in PD decreases D2 mediated inhibition of GABA transmission, which in turn results in over-activity in the D2 expressing indirect pathway (Emir *et al*, 2012; Lindfors *et al*, 1989). Simultaneously, the decreased dopamine levels observed in PD decreases binding to D1 receptors, which leads to reduced activity in the D1 expressing direct pathway of PD patients (Obeso *et al*, 2000). Together, these changes in the activity in the direct and indirect pathway increase excitation of the SNr and GPe. This in turn enhances their inhibition of the thalamus, and, ultimately, reduces the excitatory input to the motor cortex. These changes, and their ultimate inhibitory effect on the motor cortex, are believed to underlie the impaired motor function observed in PD (Figure 1.2; Wichmann & DeLong, 1996; Obeso *et al*, 2000; Blandini *et al*, 2000). Support for the hypothesis of an imbalance between the direct and indirect pathways in PD is provided, for example, by data demonstrating decreased levels of the direct pathway peptide substance P, and increased mRNA levels of the indirect pathway peptide enkephalin in the caudate of PD patients (Fernandez *et al*, 1992; Nisbeth *et al*, 1995).

Whilst the classic direct and indirect pathway model has been highly influential in the field of PD research, it should be noted that the model has been criticised for being oversimplistic. For example, contrary to the model, some striatal neurons are believed to co-express D1 and D2 receptors and a subpopulation of them also appears to project to both pathways. This challenges the view that the direct and indirect pathways are anatomically and chemically distinct (Parent *et al*, 2000). Neither does the classical model incorporate the more recently identified hyperdirect pathway. This is a cortico-subthalamic pathway that is believed to exert similar excitatory effects on the GPi and SNr as the indirect pathway, but the effects of which occur faster as the hyperdirect pathway acts directly on the GPi/SNr instead of being diverted via the striatum and GPe (Wichmann *et al*, 2011).

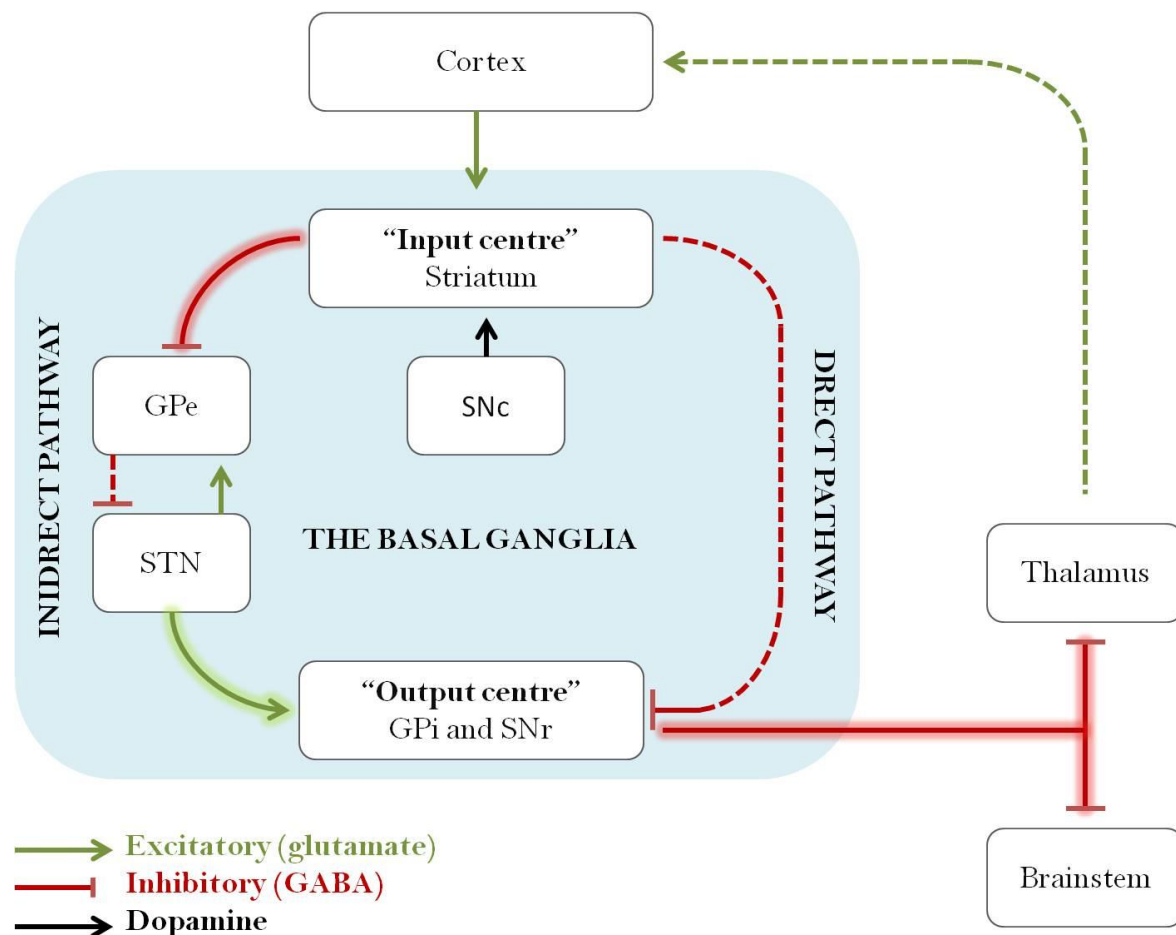


Figure 1.2. The basal ganglia in Parkinson’s disease. Shaded lined represent a PD induced increase in the strength of a connection and dotted lines a PD induced decrease in the strength of a connection. GPe=Globus pallidus external segment, GPi=Globus pallidus internal segment, SNc=Substantia nigra pars compacta, SNr=Substantia nigra pars reticulata, STN=Subthalamic nucleus.

1.4. Alpha-synuclein pathology

In addition to an imbalance between the direct and indirect pathways, PD patients also present with Lewy body pathology. Lewy bodies contain abnormal alpha synuclein aggregations as well as abnormally phosphorylated neurofilaments and ubiquitin, and are found in multiple locations in PD patients' central nervous system (Spillantini *et al*, 1998; Braak *et al*, 2003; Braak & Braak, 2000). Since the discovery of Lewy bodies in PD, these have gained increasing attention and it has been proposed that PD progression can be rated according to the spread of Lewy body pathology (Braak *et al*, 2003). The proposed rating scale divides PD progression into six steps with Lewy body pathology being restricted to the medulla oblongata in step 1, and spreading to the pontine tegmentum, the midbrain, basal procephalon & mesocortex, and neocortex in steps 2-6 respectively (Braak *et al*, 2003). This proposed model is supported by clinical data showing that alpha-synuclein pathology is capable of spreading from affected to healthy neurons (Hawkes *et al*, 2007; Li *et al*, 2008; Hansen *et al*, 2011). This ability of Lewy bodies to spread from affected to healthy neurons has also raised the question of whether or not PD is a prion disease. However, it should be mentioned that this hypothesis has been contested based on differences in the manner in which the alpha synuclein pathology spreads in PD and acknowledged prion diseases (Hiker *et al*, 2011).

The precise mechanisms underlying Lewy body formation and their involvement in dopaminergic cell death are still unknown. It has been suggested that the alpha-synuclein aggregations interact with dopamine and mitochondrial activity to increase reactive oxygen species, which in turn leads to cell death (Perez *et al*, 2002; Maguire-Zeiss *et al*, 2005). A causal role of Lewy bodies in cell death has been suggested by preclinical data showing impaired motor function, decreased striatal dopaminergic terminals, and Lewy Body resembling inclusions in the cortex, hippocampus, and SNc of transgenic mice over-expressing alpha synuclein (Masliah *et al*, 2000). However, with later publications failing to replicate these findings (Matsuoka *et al*, 2011; Rathke-Hartlieb *et al*, 2001), it has yet to be determined whether the Lewy bodies observed in PD are indeed causal for the dopaminergic cell death, secondary to another pathological feature of the disease, or part of the central nervous system's protective response (Maguire-Zeiss *et al*, 2005; Ross & Poirier, 2005).

1.5. Risk factors for Parkinson's disease

1.5.1. Genetic risk factors

PD is not considered to be a monogenetic disorder. However, current data suggest that mutations to parkin, and PTEN-induced putative kinase 1 (PINK1), and leucine-rich repeat kinase 2 (LRRK2) all increase the risk for developing PD (Mata *et al*, 2006; Lücking *et al*, 2000; Valente *et al*, 2004a).

The parkin gene, first described by Kitada and colleagues (1998), has been associated with early onset PD. One cohort study identified a parkin mutation in 18% of participants diagnosed with PD, and in 49% of non-diagnosed relatives. Amongst the PD patients included in the study, the parkin mutation was most common in individuals with young-onset PD (Lücking *et al*, 2000). Parkin is believed to contribute to PD via its involvement in the cellular response to mitochondrial damage (Narendra *et al*, 2008) and the ubiquitin proteasome system (UPS) (Sherman & Goldberg, 2001; Betarbet *et al*, 2005). Post-mortem data have demonstrated that the UPS system is impaired in the SNc of PD patients (McNaught *et al*, 2003). Parkin interacts with the UPS system by ubiquinating damaging proteins, such as misfolded alpha synuclein, that are targeted for UPS-mediated degradation and thereby aids their removal (Sherman & Goldberg, 2001; Betarbet *et al*, 2005). Following UPS inhibition, *in vitro* data have shown increased alpha synuclein inclusions in ventral mesencephalic neurons and decreased survival of dopaminergic neurons (McNaught *et al*, 2002). Together, the data therefore suggest that mutations to parkin may increase predisposition to PD both by impairing mitochondrial function and by inducing dysregulation of proteasome function, which may ultimately contribute to the alpha synuclein pathology and dopaminergic cell loss that is characteristic of PD (Spillantini *et al*, 1998; Braak & Braak 2000).

Parkin is believed to act downstream of another gene also associated with PD; PINK1 (Clarke *et al*, 2006). Like parkin, PINK1 is associated with early onset PD with one study demonstrating PINK1 mutations in 7% of patients diagnosed prior to the age of 50 (Valente *et al*, 2004a). The gene is believed to be part of the cellular response to stress, with cells expressing the mutation showing mitochondrial dysfunction and apoptosis when exposed to oxidative stress (Valente *et al*, 2004b).

PD is also associated with mutations to LRRK, which interacts with the parkin gene (Smith *et al*, 2005). LRRK2 mutations are believed to be present in 6-40% of familial and approximately 3% of sporadic PD cases, depending on ethnicity (Mata *et al*, 2006), and are

predominantly associated with late onset PD (Zimprich *et al*, 2004). Mutations to the gene have been shown to increase the percentage of human neuroblastoma SH-SY5Y cells undergoing apoptosis in response to cellular stress, a phenomenon which is believed to be caused by a LRRK2 interaction with the caspase 3, mitochondria dependent, apoptosis pathway (Iaccarino *et al*, 2007). Furthermore, data from aged LRRK2 knockout mice have shown that loss of LRRK increases the levels of ubiquitinated proteins and alpha synuclein in the kidneys, which are known to have high concentration of LRRK2 mRNA relative to other organs (Tong *et al*, 2010). This has led to the hypothesis that LRRK2 mutations, like Parkin mutations, contribute to the alpha synuclein pathology observed in PD patients by disrupting the UPS system (Tong *et al*, 2010).

Whilst only a minority of individuals expressing LRRK2, parkin, or PINK1 mutations develop PD, the cited studies suggest that mutations affecting mitochondrial function and the UPS system may increase the risk of developing PD.

1.5.2. Environmental risk factors

In addition to genetic predispositions, clinical and preclinical studies have also identified a range of environmental risk factors that are believed to increase the risk for PD. The most prominent environmental risk factor is exposure to specific herbicides or insecticides (Gorell *et al*, 1998). Two toxins that have received particular attention are rotenone and paraquat. Systemic administration of the pesticide rotenone has been shown to cause mitochondrial complex I inhibition, impair proteasomal function, induce alpha-synuclein inclusions in dopaminergic neurons, cause loss of dopamine neurons, and cause motor deficits in rats (Betarbet *et al*, 2000; Derek & Patel, 2008). Similarly, systemic administration of the herbicide paraquat has been shown to decrease survival of dopaminergic neurons and impair locomotor activity, as well as inducing alpha-synuclein aggregations in mice (Brooks *et al*, 1999; Manning-Bog *et al*, 2002).

The studies cited in this and the previous (1.5.1) section demonstrates the presence of both genetic and environmental risk factors for PD. With neither being able to account for all cases of PD, the cause of the disease remains unknown. Instead, it is yet to be determined whether PD arises from an interaction between genetic and environmental factors, or if genetic and environmental risk factors give rise to different subtypes of the disease.

1.6 Experimental models of Parkinson's Disease

Whilst PD is not known to occur spontaneously in other species, aspects of its pathology may be mimicked in preclinical models. Commonly used models capture the alpha synuclein pathology observed in PD, genetic mutations linked to the disease (e.g. LRRK2, PINK1, and Parkin), or induce dopamine loss via administration of pharmacological agents or neurotoxins. While there is a range of preclinical models of PD available (reviewed in e.g. Duty & Jenner, 2011), the following sections will describe some of the most common preclinical models before focusing on the 6-hydroxy-dopamine (6-OHDA) rat model which was used in this thesis project, and its use in dyskinesia research.

1.6.1. Models of alpha-synuclein pathology

In addition to SNc dopamine loss, PD is characterised by the presence of Lewy bodies. Findings of alpha-synuclein aggregations within Lewy bodies (Spillantini *et al*, 1997), and an association between mutations to the alpha synuclein gene and familial PD (Polymeropoulos *et al*, 1997) encouraged the development of preclinical models mimicking the alpha synuclein pathology observed in PD. In 2000, Mashlia and colleagues demonstrated that over-expression of wild-type human alpha-synuclein in mice caused a loss of striatal dopamine and motor deficits. Unfortunately, the effect on motor function has not always been replicable, limiting the use of this specific model in studies on the effect of alpha synuclein on motor function (Matsuoka *et al*, 2011; Rathke-Hartlieb *et al*, 2001). However, in addition to motor deficits, a separate line of research has found that transgenic mice over-expressing alpha-synuclein exhibit hyposmia (Fleming *et al*, 2008), an important non-motor symptom of the disease (Haehner *et al*, 2009; Politis *et al*, 2010). Hence, transgenic models of alpha synuclein over-expression may be useful for studying the effect of alpha synuclein inclusions on certain non-motor symptoms of PD.

As an alternative to transgenic models, the alpha-synuclein pathology observed in PD may also be mimicked by stereotactic infusion of viral vectors expressing mutated alpha-synuclein. This has been shown to induce alpha-synuclein aggregations, loss of dopaminergic neurons, and impaired motor performance in rats with a SNc dopamine loss of approximately 60% (Lo-Bianco *et al*, 2002; Kirik *et al*, 2002). Whilst not widely used, infusion of vectors carrying human alpha-synuclein has also been combined with rotenone exposure to produce a model exhibiting greater motor impairments (Mulcahy *et al*, 2012).

1.6.2. Models of genetic risk factors

As described in section 1.5.1, the LRRK2, parkin, and PINK1 genes have all been linked to PD. These genes have been studied in a range of preclinical models, including transfected cell lines (e.g. Iaccarino *et al*, 2007; Narendra *et al*, 2008; Valente *et al*, 2004b), *drosophila* (e.g. Liu *et al*, 2008; Poole *et al*, 2008; Yang *et al*, 2006), and mice (e.g. Li *et al*, 2009; Itier *et al*, 2003; Gispert *et al*, 2009).

Over-expressing LRRK2 in *drosophila* affects mitochondria function, as indicated by an increased sensitivity to the mitochondria complex I inhibitor rotenone (Ng *et al*, 2009). Furthermore, over-expression of human and mutant LRRK2 in *drosophila* causes a loss of dopaminergic neurons and motor deficits such as reduced climbing ability and spontaneous locomotor activity (Ng *et al*, 2009; Liu *et al*, 2008). The climbing deficit can be alleviated by acute administration of the dopamine precursor L-DOPA (Liu *et al*, 2008), suggesting that the LRRK2 *drosophila* may be used for screening antiparkinsonian drugs. LRRK2 has also been overexpressed in transgenic mouse models, where it has been shown to increase alpha synuclein aggregation, induce neuronal loss in the prefrontal cortex and dorsal striatum, and decrease spontaneous activity and rearing behaviour (Lin *et al*, 2009). Similar to the *drosophila* model, the reduced spontaneous motor activity in transgenic mice has been shown to improve following acute L-DOPA treatment (Li *et al*, 2009).

The PINK1 gene has also been studied in transgenic models of PD. In *drosophila*, inactivation of PINK1 impairs mitochondrial activity, induces loss of dopaminergic neurons, and reduces flight ability (Yang *et al*, 2006; Park *et al*, 2006). Mitochondrial dysfunction has also been observed in a PINK1 transgenic mouse model (Gispert *et al*, 2009). While, unlike the *drosophila*, these transgenic mice did not show a loss of dopaminergic neurons, they exhibited decreased striatal dopamine levels and a reduction in their spontaneous locomotor activity - changes which were hypothesised to be caused by disrupted dopamine neurotransmission (Gispert *et al*, 2009). It is uncertain whether the motor symptoms expressed by PINK1 transgenic mice can be reversed or ameliorated by L-DOPA, as is the case in other transgenic models (Dawson *et al*, 2010), although there are indications that L-DOPA treatment may restore abnormal spontaneous GABA currents that are observed in PINK1 knockout mice (Dehorter *et al*, 2012).

Transgenic models of parkin have also been developed. In *drosophila*, loss of parkin function reduces dopamine levels, and causes a climbing deficit which can be ameliorated by acute L-DOPA administration (Cha *et al*, 2005). Conversely, *over-expressing* parkin in *drosophila* may alleviate or rescue the detrimental effects of other PD related mutations. For

example, in transgenic LRRK2 *drosophila*, co-expression of parkin reduces both the LRRK2 induced degeneration of dopaminergic neurons and the neurodegenerative effect of rotenone exposure (Ng *et al*, 2009). Similarly, in transgenic *drosophila* with inactivated PINK1, over-expression of parkin rescues mitochondria function, reduces the otherwise observed loss of dopaminergic neurons, and reverses the flies' climbing deficit (Yang *et al*, 2006; Park *et al*, 2006).

The effect of parkin on PD symptoms has also been modelled in knock-out mice. Whilst not showing a loss of dopaminergic neurons, parkin knock-out mice exhibit changes indicative of disruptions to the dopaminergic system such as an increase in limbic dopamine levels without an associated change in dopamine turnover, and decreased DAT and VMAT levels (Itier *et al*, 2003). Similar findings have been reported by other groups, who have shown that parkin knock-out mice have higher striatal dopamine levels than wildtype controls (Goldberg *et al*, 2003). These data have been suggested to reflect impaired control of dopamine release from nigral neurons (Itier *et al*, 2003; Goldberg *et al*, 2003). On a behavioural level, transgenic mice show impaired motor function, such as reduced spontaneous motor activity, making more slips than controls when walking across an elevated beam, and somatosensory impairments (Itier *et al*, 2003; Goldberg *et al*, 2003), as well as a decreased motor response to acute amphetamine administration (Itier *et al*, 2003).

1.6.3. The MPTP model

Neurotoxins are commonly used in the preclinical setting to induce a dopamine loss resembling the decreased dopamine levels observed in PD. One of the most commonly used toxins is MPTP.

In the late 1970's and early 1980's, a number of American drug addicts were found with severe movement disruptions. Whilst the symptoms resembled those observed in PD, their rapid onset and the young age of the patients were uncharacteristic of idiopathic PD. Instead, it became apparent that the patients had previously self-administered what they had believed to be the recreational drug 1-Methyl-4-phenyl-4-propionoxypiperidine (MPPP) but which had, due to poor synthesis, in fact been its precursor MPTP. Within one week of MPTP administration, the patients began exhibiting akinesia, rigidity, and difficulties speaking (Langston *et al*, 1983). Improvement of the symptoms following administration of the anti-Parkinsonian drug L-DOPA and a D2 agonist suggested that the akinesia was caused by a dopamine deficiency, something which was confirmed by PET imaging and post-mortem examination (Davis *et al*, 1979; Langston *et al*, 1983).

It is now known that MPTP is taken up by astroglia, where it is converted into 1-methyl-4-phenyl-2,3-dihydropyridinium cation (MDPD⁺) and ultimately 1-methyl-4-phenylpyridinium ion (MPP⁺) by monoamine oxidase (MAO). MPP⁺ is taken up by dopaminergic nerve terminals where it accumulates. Once inside the neurons, the toxin is believed to induce cell death by inhibiting mitochondrial activity, as well as by increasing intraneuronal calcium to toxic levels (Gerlach *et al*, 1991). In line with the case studies presented by Langston and colleagues (1983), systemic administration of MPTP has been shown to induce profound loss of dopamine, its metabolites, and serotonin in experimental settings (Gerlach *et al*, 1991; Johnston *et al*, 2010a; Lundblad *et al*, 2005).

Today, MPTP is widely used in preclinical PD research. It is most commonly used to induce dopamine loss in mice and primates. It is not typically used in rats, where the central nervous system is able to clear MPTP and its metabolites more rapidly than in mice (Pileblad *et al*, 1985; Johannessen *et al*, 1985), and the effect of MPTP is therefore more variable. The MPTP induced motor symptoms may be alleviated by acute L-DOPA administration (Johnston *et al*, 2010a; Lundblad *et al*, 2005). Furthermore, long-term L-DOPA exposure in MPTP lesion mice and primates causes the onset of LID, a common side effect of long-term L-DOPA treatment in patients that is further described in section 1.8.3. In MPTP models, LID may be reduced by the clinically available anti-dyskinetic drug Amantadine (Blanchet *et al*, 1998; Lundblad *et al*, 2005), suggesting MPTP models are valid preclinical model of LID. In addition to motor symptoms, MPTP has been used to mimic non-motor symptoms of PD such as reduced REM sleep (Barraud *et al*, 2009), constipation (Natale *et al*, 2010), hyper-reflexive bladders that result in more frequent spontaneous emptying of bladders than in healthy animals (Albanese *et al*, 1988), and cognitive deficits in the form of impaired delayed alternation (Schneider & Kovelowski, 1990).

Whether MPTP administration can mimic the alpha synuclein pathology observed in PD remains under debate with such inclusions being reported in some but not all MPTP studies and, when present, being qualitatively different from human Lewy bodies (Meredith *et al*, 2002; Vila *et al*, 2000; Shimoji *et al*, 2005).

1.6.4. The 6-hydroxy-dopamine model

In rats, PD motor symptoms are most commonly induced by infusing the neurotoxin 6-OHDA along the nigrostriatal pathway: typically the SNc, striatum, or the medial forebrain bundle (MFB). The applicability of 6-OHDA to PD research was first recognised in the late 1960's, when Ungerstedt (1968) discovered that infusion of the toxin into dopamine rich

regions caused dopaminergic and noradrenergic cell death. 6-OHDA is believed to induce cell death via at least two separate routes. First, inhibition of complex I disrupts the mitochondrial respiratory chain, which in turn is believed to disrupt the mitochondrial membrane potential and increase reactive oxygen species (ROS). Second, it is believed that further interaction between 6-OHDA and MAO causes auto-oxidation of the toxin, as well as generating damaging ROS (Glinka & Youdim, 1995; Blum *et al*, 2001).

6-OHDA is unable to cross the blood brain barrier and is therefore delivered to the neural region of interest via stereotaxic surgery. 6-OHDA lesions are commonly only induced unilaterally, as bilateral dopamine denervation is associated with aphagia, adipsia, and weight loss (Zigmond & Stricker, 1972; Sakai & Gash, 1994). Following unilateral infusion to the dopaminergic system a hemi-Parkinsonian model is created, where akinesia is predominantly exhibited in the limbs contralateral to the lesion (e.g. Whishaw *et al* 1997, Olsson *et al*, 1995). The 6-OHDA lesion has also been used to study non-motor effects of dopamine denervation such as sensorimotor functions and the accuracy with which lesion rats respond to a conditioned stimulus (CS) in a lateralised choice-reaction time task (Carli *et al*, 1985; Dowd & Dunnett, 2004, 2007), which is further described in section 1.6.7.1.

The akinesia exhibited in the 6-OHDA model can be alleviated by common antiparkinsonian drugs such as L-DOPA, dopamine agonists, and MAO inhibitors (Lundblad *et al*, 2002; Marin *et al*, 2007; MacInnes & Duty, 2004; Olsson *et al*, 1995; Lindgren *et al*, 2007). As described in the following section, the model is also widely used to study LID.

1.6.5. Modelling dyskinesia in the 6-hydroxy-dopamine model

PD motor symptoms are commonly treated by acute administration of the dopamine precursor L-DOPA. However, as further discussed in section 1.8.3, long-term L-DOPA treatment often leads to onset of the motor side effect LID. LID may also be induced in 6-OHDA lesion rats, where the dyskinetic movements are referred to as abnormal involuntary movements (AIMs).

Repeated administration of L-DOPA causes a gradual development of AIMs in the majority of rats with extensive 6-OHDA lesions to the dopaminergic system (Winkler *et al*, 2002). The AIMs manifest themselves when lesion rats are on L-DOPA, and their magnitude plateau after approximately 2 weeks of daily injections, depending on dose (e.g. Lundblad *et al*, 2002; Winkler *et al*, 2002). The dyskinetic movements exhibited in the rat model include axial AIMs characterised by a twisting of the torso towards the side contralateral to the lesion, that in severe cases causes a loss of balance (Figure 1.3A), forelimb AIMs that are

manifested in the form of purposeless movements of the forelimb contralateral to the lesion (Figure 1.3B), and orolingual AIMs which include either a chewing motion or in more severe cases protrusions of the tongue (Figure 1.3C ; Cenci *et al*, 1998; Winkler *et al*, 2002).



Figure 1.3. Stillshots of a rat with a unilateral 6-OHDA lesion exhibiting axial (a), forelimb (b), and orolingual (c) AIMs following an acute injection of L-DOPA. AIMs=Abnormal involuntary movements. Reprinted from Winkler *et al*(2002).

The AIMs expressed by rats with unilateral 6-OHDA lesions can be measured using different rating scales (Breger *et al*, 2013). The original rat AIMs scoring protocol was developed by Cenci and colleagues (1998) and classified movements as “abnormal” if they were (i) L-DOPA induced, (ii) affected the side of the body contralateral to the 6-OHDA lesion, and (iii) were repetitive, purposeless, and not exhibited in the normal rat behavioural repertoire. Cenci *et al* (1998) scored rats for 1 min, every 20 min, and rated axial, forelimb, and orolingual AIMs on a scale from 0 to 4 depending on the length of time the AIM was exhibited during each 1 min scoring point (0=never, 1=less than 50% of the time, 2=more than 50% of the time, 3=continuously but could be interrupted by sudden noises, 4=continuously and could not be interrupted by sudden noises; Cenci *et al*, 1998). In addition, Cenci and colleagues (1998) rated locomotive AIMs, classified as an increased locomotive behaviour towards the contralateral side, using the same point scale. However, since the original scale was described, later publications have queried the validity of the locomotive AIMs. Locomotive AIMs are not reduced by drugs that have anti-dyskinetic effects in the clinic, and that reduce other rat AIMs subtypes (Lundblad *et al*, 2002), thus suggesting that they are not a good model for human LID. The original rating scale has also been extended by Winkler and colleagues (2002) to score AIMs according to their severity as well as the duration, thereby increasing the sensitivity of the scale.

Since its development, the validity of the 6-OHDA rat dyskinesia model has been supported by data showing that (i) just as in human LID, rat AIMs are induced by L-DOPA

but not D2 agonists, (ii) just as in human LID, there is a correlation between AIMs and FosB/ Δ FosB upregulation, and (iii) that AIMs decrease following administration of the only clinically available anti-dyskinetic drug, Amantadine (Lundblad *et al*, 2002; Andersson *et al*, 1999; Lindgren *et al*, 2011; Breger *et al*, 2013).

1.6.6. Testing motor function in the unilateral 6-OHDA lesion model

To provide a background to the experimental chapters presented in this thesis, the following sections will provide an overview of behavioural tests that were used in the thesis project to study motor and non-motor function in the unilateral 6-OHDA rat model of PD. Further information on the manner in which the tests were conducted is provided in Chapter 2.

1.6.6.1. Rotational bias as a measurement of dopamine denervation

When first describing the effect of 6-OHDA infusion on dopaminergic cells, Ungerstedt (1968) reported that rats with unilateral lesions to the dopaminergic system spontaneously turned towards the side of the lesion. The degree of turning behaviour was related to the extent of dopaminergic denervation, and driven by asymmetry between dopamine levels in the intact and lesion striata (Ungerstedt, 1968). The finding was replicated in a subsequent study which also demonstrated that the rotational bias could be enhanced by acute administration of amphetamine (Ungerstedt & Arbuthnott, 1970); a drug which causes release of presynaptic dopaminergic vesicles whilst inhibiting DAT activity and thus increases striatal dopamine levels (Sulzer *et al*, 2005). Following unilateral 6-OHDA lesions, fewer dopaminergic terminals are present in the lesion than the intact striata, which causes the vast majority of amphetamine induced dopamine release to occur in the intact striata. This exacerbates the dopamine imbalance between the hemispheres, and thereby enhances the ipsilateral rotational bias that is observed following unilateral dopamine depletion (Ungerstedt, 1968; Ungerstedt & Arbuthnott, 1970). Acute amphetamine may induce a rotational bias in rats with a striatal dopamine loss as low as 50% relative to the intact striata, although not all lesion rats rotate at this level of depletion (Hefti *et al*, 1980). Amphetamine induced rotations are commonly used post-lesion to assess the success of 6-OHDA lesions, with pre-determined cut-off points often being applied to identify rats with extensive dopamine loss (Fornaguera *et al*, 1994). The test has been used to screen lesion success following unilateral 6-OHDA lesions to the SNc (e.g. Ungerstedt & Arbuthnott, 1970), the striatum (e.g. Barneoud *et al*, 2000), and the MFB (e.g. Torres *et al*, 2011).

However, despite its widespread use, the amphetamine induced rotational bias does not correlate well with fine motor function (Metz & Whishaw, 2002). Whilst the reason for

this poor correlation has not been firmly concluded, it has been suggested that the SNc is organised in a somatotopographical manner and that lesions affecting e.g. skilled forelimb motor function do not affect whole body rotational behaviour (Metz & Whishaw, 2002). Therefore, crude pharmacological tests, such as amphetamine induced rotations, that are sensitive to large dopamine denervation might not be suitable for predicting skilled motor function and are of limited use when wishing to balance experimental groups in studies assessing finer motor skills. Further concerns regarding the value of amphetamine induced rotations as a lesion screening tool stem from findings that amphetamine exposure alters, for example, c-fos mRNA levels which are implicated in learning (Cadet *et al*, 2010), that it affects later neurochemical and motor responses to dopaminergic drugs (Vanderschuren *et al*, 1999), and that it predisposes animals to acquire habitual rather than goal-directed behaviour when tested on operant tasks (Nelson & Kilcross, 2006; Nordquist *et al*, 2007).

Other anti-parkinsonian drugs such as L-DOPA and dopamine agonists have also been shown to induce a rotational bias in the unilateral 6-OHDA model (e.g. Hefti *et al*, 1980; Barneoud *et al*, 2000; Lundblad *et al*, 2002). The lesion striatum is less able to store the dopamine which is synthesised from L-DOPA than the intact striatum, and acute L-DOPA administration therefore causes greater extracellular dopamine levels in the lesion than intact striata, resulting in a contralateral rotational bias (Smith *et al*, 2010). Dopamine agonists such as apomorphine and bromocriptine cause contralateral turning by binding to postsynaptic receptors which are sensitised in the lesion but not the intact striatum (Hefti *et al*, 1980; Barneoud *et al*, 2000; Lundblad *et al*, 2002). However, despite their ability to induce a rotational bias post-lesion, the use of L-DOPA and dopamine agonists is not without its limitations when screening for 6-OHDA lesions. Repeated L-DOPA administration induces LID in the majority of 6-OHDA lesion rats (e.g. Winkler *et al*, 2002), and L-DOPA induced rotations are therefore inappropriate as a screening tool for studies comparing the behaviour of L-DOPA treated and drug naive rats, such as the experiments reported in Chapters 6-8. Apomorphine, the dopamine agonist most commonly used to screen 6-OHDA lesion success sensitises dopamine receptors (Gancher *et al*, 1995), and is not sensitive to dopamine loss <90% (e.g. Heft *et al*, 1980). The latter makes it an inappropriate screening method for lesions inducing a lesser depletion, such as the intrastriatal 6-OHDA lesions reported in Chapters 4 and 5.

1.6.6.2. Testing motor function in the unilateral 6-OHDA rat

Following unilateral 6-OHDA lesions, the lesion induced motor impairments predominantly manifest themselves in limbs contralateral to the lesion. The discrepancy in ipsilateral and contralateral motor ability can be measured using a range of behavioural tests. Commonly used tests of motor asymmetry include the cylinder test, the adjusting step test, the vibrissae test, and the staircase test.

The cylinder test (section 2.4.4) measures rats' spontaneous use of their ipsilateral and contralateral forelimbs when exploring a novel environment consisting of a transparent cylinder. During explorative behaviour, rats often rear up against the wall of the cylinder and, when doing so, commonly use their forelimbs to support themselves. Intact rats will use both their forelimbs to an equal extent, while lesion rats predominantly support themselves using their ipsilateral forelimb (Schallert *et al*, 2000). By measuring the extent to which rats use their ipsilateral and contralateral forelimbs during explorative behaviour, the cylinder test provides a measurement of the motor asymmetry induced by a unilateral lesion. While the test is sensitive to dopaminergic treatments such as L-DOPA and the D2 agonist bromocriptine (Lundblad *et al*, 2002), it is dependent on spontaneous explorative behaviour. This explorative behaviour may decrease following repeated exposure to the cylinder which limits the number of times the test can be used in the same animal.

This problem is overcome in tests where movement is directly encouraged by the experimenter. Examples of such tests are the adjusting step (section 2.4.3) and vibrissae tests (section 2.3.8), which measure forelimb motor function and sensorimotor function respectively (Olsson *et al*, 1995; Woodlee *et al*, 2005). Both tests involve gentle restraint of the rat, followed by tactile stimulation of a forelimb or vibrissae, and recording of the behavioural response this stimulation evokes. In the adjusting step test the experimenter restrains either the ipsilateral or contralateral forelimb while simultaneously ensuring that the free forelimb is in gentle contact with a flat tabletop surface. The rat is then moved along the tabletop surface and the number of stepping motions the rat initiates using its free forelimb is measured. The same procedure is repeated following restriction of the other forelimb, and the number of adjusting steps a rat makes using the forelimb ipsilateral and contralateral to the lesion are compared (Woodlee *et al*, 2005).

As in the adjusting step test, the vibrissae test also involves restraint of the forelimb ipsilateral or contralateral to the lesion by the experimenter. The rat is then moved in an upwards direction parallel to a bench top, so as to cause the rat's ipsilateral or contralateral whiskers to brush against the bench top. The test measures rats' ability to respond to whisker

stimulation by placing their free forelimb on the bench, and compares the number of responses made following stimulation of their ipsilateral and contralateral whiskers (Olsson *et al*, 1995).

The above tests offer a measurement of crude motor function. Other behavioural tasks may be used in instances where finer motor skills are measured. These include qualitative rating of animals' movements (reviewed in Klein *et al*, 2012), and the staircase test (Montoya *et al*, 1991). The latter measures rats' and mice' ability to grasp and retrieve sucrose pellets in so called 'staircase boxes' when using the forelimb ipsilateral or contralateral to the lesion (section 2.4.7). Unlike handtests, such as the adjusting step test and the vibrissae tests, or subjective rating scales, the staircase test holds the advantage of being free from experimenter bias. The movement sequence involved in picking up an object using a forelimb is highly similar between rats and people (Sacrey *et al*, 2009), suggesting that drug-induced improvement on the staircase and other reaching tasks may predict therapeutic relief in the clinic.

1.6.7. Testing operant behaviour in the unilateral 6-OHDA lesion model

Operant boxes are commonly used to measure cognitive functions in preclinical rat models as they remove experimenter bias, are capable of recording a range of parameters simultaneously, and may be programmed to be used for a variety of paradigms. A range of operant boxes are commercially available, including the nine-hole box (section 2.7.2; Robbins *et al*, 1993) where animals may respond to visual or auditory stimuli by nose-poking into an array of holes, and the two-lever 'Skinner box' chamber (section 2.7.1, e.g. Döbrössy & Dunnett, 1997) where animals respond to visual or auditory stimuli by pressing lateralised levers.

The majority of operant box tasks are designed for either intact rats, or rats with bilateral lesions. However, this thesis project used a unilateral 6-OHDA rat model of PD. To study the effects of acute and chronic dopaminergic medication on motor and non-motor function, it was therefore necessary to identify an operant paradigm which was (i) suitable for rats with unilateral lesions and (ii) able to dissociate between the effects dopamine denervation on motor and non-motor behaviour. One such task is the lateralised choice reaction time (LCRT) task which takes place in nine-hole operant boxes and is described in more detail below.

1.6.7.1. The lateralised choice reaction time task

The lateralised choice reaction time (LCRT) task (section 2.7.2.2) was originally developed by Carli and colleagues (1985) to distinguish between visual neglect, movement initiation, and movement execution. The task requires rats to respond to illumination of a central hole by making, and maintaining, a centralised nose poke until a visual CS is presented on either the ipsilateral or contralateral side (Carli *et al*, 1985). In response to the CS, rats must make a lateralised nose poke to gain a pellet reward (Carli *et al*, 1985). Depending on the version of the LCRT task that is used, this response is either directed to the same hole in which the CS was presented ('same' version) or the opposite lateralised hole ('opposite' version; Figure 1.4).

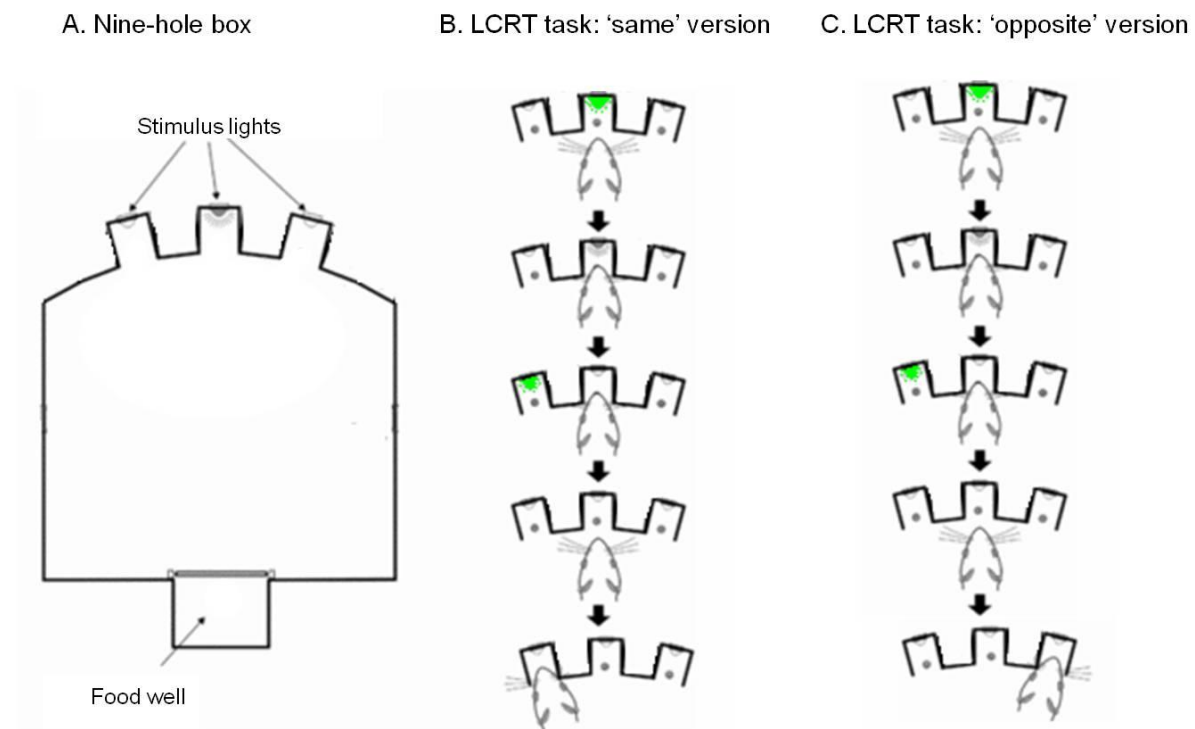


Figure 1.4. A schematic overview of a nine-hole operant box with only three response holes are exposed as is the case in the LCRT task (A), and an example of correct responding in the 'same' (B) and 'opposite' (C) versions of the LCRT task. The rats are rewarded for responding to a central light cue by making a sustained nose poke in the central hole until the onset of a lateralised CS, upon which they must either respond in the same response hole the CS was presented in ('same' version, B) or in the opposite response hole ('opposite' version, C). Adapted from Dowd & Dunnett, 2005. CS=Conditioned stimulus; LCRT=Lateralised choice reaction time

The task provides four main outcome measures: usable trials, reaction time, movement time, and accuracy. ‘Usable trials’ refer to the proportion of trials that rats complete by executing a lateralised nose poke in response to the lateralised CS. ‘Reaction time’ refers to the time elapsed between the onset of a lateralised CS and discontinuation of the central nose poke. ‘Movement time’ refers to the time elapsed between discontinuation of a central nose poke and the completion of a subsequent lateralised nose poke. Finally, ‘accuracy’ refers to the proportion of usable trials in which rats direct their lateralised responses into the correct lateral hole following presentation of a lateralised CS.

Using the LCRT task, Carli and colleagues (1985) found that rats that had been pre-trained on the task prior to unilateral 6-OHDA lesions to the striatum exhibited increased reaction time and decreased accuracy when required to respond to contralateral CS by making contralateral nose pokes (‘same’ version of the task). No deficit was observed when rats were required to respond to ipsilateral CS by making ipsilateral nose pokes (Carli *et al*, 1985). A reaction time and accuracy deficit did, however, show when rats were tested on the ‘opposite’ version of the task which required them to respond to contralateral CS by making ipsilateral nose pokes and vice versa. In this version of the test, the deficit manifested itself when rats were required to execute contralateral responses to ipsilateral cues. Hence, the deficit was not related to the position of the visual stimulus but rather the direction of the required motor response. Furthermore, when altering the task so that the rats’ response to a visual cue consisted of making a full turn and pressing the panel on the opposite wall, thus allowing them to respond to contralateral cues by either an ipsilateral or a contralateral turn, the accuracy deficit that had previously been observed following 6-OHDA lesions disappeared (Carli *et al*, 1985). Together, the data suggested that the deficit was not due to an inability to detect the lateralised CS, but an impaired ability to initiate or execute contralateral movements following 6-OHDA lesions (Carli *et al*, 1985).

The neurobiology of the deficit was explored in a subsequent study which compared the effects of 6-OHDA lesions to the nucleus accumbens (NAcc) and the striatum on performance on the ‘same’ version of the LCRT task (Carli *et al*, 1989). The previous findings of a 6-OHDA induced deficit in contralateral reaction time and accuracy (Carli *et al*, 1985) were replicated in rats with intrastriatal lesions but not in the NAcc lesion group (Carli *et al*, 1989). This led the authors to hypothesise that dorsal striatal dopamine is essential for rats’ ability to initiate contralateral movements (Carli *et al*, 1989).

Based on the findings of Carli *et al* (1985, 1989), the LCRT task was later employed by Dowd & Dunnett (2004) as a means of studying the behavioural effects of ventral

mesencephalic tissue transplants on rats with unilateral 6-OHDA lesions to the MFB. In line with Carli and colleagues' original findings (1985, 1989), dopamine depletion impaired reaction time and accuracy when rats responded to contralateral cues by making contralateral responses ('same' version). In addition, there was a bilateral increase in movement time that had not previously been reported. The appearance of a movement time deficit may have been due to the greater dopamine depletion induced by MFB lesions, relative to the intra-striatal lesions used by Carli and colleagues (1985, 1989). It may also have been due to the distance between the centre hole and the lateralised hole in which rats' responded to a CS, which was greater in Dowd & Dunnett's (2004) than in Carli and colleagues' (1985, 1989) experiments.

Dowd & Dunnett's (2004) publication also differed from Carli and colleagues' (1985, 1989) in the manner in which they illustrated the post-lesion behaviour. Whereas the publications by Carli and colleagues (1985, 1989) presented bar graphs showing the post-lesion performance averaged across a block of testing, Dowd & Dunnett (2004) used line graphs to show the post-lesion behaviour as a function of time. When presenting the contralateral accuracy using a line graph it became apparent that the contralateral accuracy deficit manifested itself gradually (Dowd & Dunnett, 2004). Specifically, the lesion group was able to accurately respond to contralateral stimuli on the first day of post-lesion testing but thereafter displayed a gradual decrease in contralateral accuracy over the subsequent four days of testing. Interestingly, following a break from testing and reintroduction to the task eight weeks post-lesion, the accuracy of contralateral responding on the first day of testing returned to the initial post-lesion levels. Similar to what had previously been observed a gradual decline in contralateral performance was then observed over the following four days of testing. This phenomenon could not be explained by a continued loss of striatal dopamine since the lesion was complete at the time of testing. Furthermore, if the deficit was purely motor mediated the impairment would be expected to have been stable from the outset of post-lesion testing. Hence, the accuracy deficit could not be explained by Carli and colleagues' (1985, 1989) original hypothesis of an impaired ability to initiate contralateral movements (Carli *et al*, 1989; Brown & Robbins, 1989)

The finding of a gradual decline in performance rather than a stable impairment was replicated in a subsequent experiment which compared the effects of unilateral 6-OHDA intra-striatal and MFB lesions on performance on the 'same' version of the LCRT task (Dowd & Dunnett, 2005a). Both lesions caused a gradual impairment in contralateral reaction time, movement time, and accuracy when tested two weeks post-lesion, although the accuracy deficit was more pronounced in the MFB lesion group. When re-tested four months

post-lesion the intra-striatal lesion group seized showing a deficit in their reaction time, movement time, and accuracy while the MFB lesion group continued being impaired on all parameters. Interestingly, in line with previous findings, the contralateral accuracy of the MFB lesion returned to initial post-lesion testing levels on the first day of testing after which a second gradual decline in performance was observed (Dowd & Dunnett, 2005a). A follow-up experiment, including rats with MFB or intra-striatal lesions, was conducted in which the ‘opposite’ version of the LCRT task was used, i.e. where rats were required to respond to ipsilateral CS by making a contralateral response and vice versa. In this version of the task, both the MFB and intra-striatal lesion groups exhibited impaired accuracy when required to respond to ipsilateral stimuli by making a contralateral response (Dowd & Dunnett, 2005b). In contrast to the earlier findings, which were obtained using the standard ‘same’ version of the task (Dowd & Dunnett, 2005a), this accuracy deficit remained stable in both lesion groups when re-tested four months post-lesion (Dowd & Dunnett, 2005b).

From a methodological perspective, the described studies suggest that the standard ‘same’ version of the LCRT task is appropriate for long-term experiments including rats with lesions to the MFB, but not to the striatum. The difference between the two lesions’ effect on LCRT task performance may be due to the extent of dopamine denervation they induce in the SNc or VTA, which is greater following a MFB than intra-striatal lesion, or the ability of the MFB lesions to also affect prefrontal dopamine levels (Dowd & Dunnett, 2005a).

From a theoretical perspective, the most notable difference between Carli and colleagues’ (1989, 1989) and Dowd & Dunnett’s (2004, 2005a,b) publications was the demonstration of a gradually emerging contralateral accuracy deficit in the latter publications. In Dowd & Dunnett’s (2004, 2005a,b) publications, this deficit disappeared on the first day of re-testing rats following a three months test-free interval. Dowd & Dunnett (2007) noted that this re-emergence of contralateral responding resembled ‘spontaneous recovery’ of contralateral accuracy. ‘Spontaneous recovery’ is one of the hallmarks of extinction. It refers to the tendency of a conditioned response (CR) to reappear in animals that have undergone extinction when these animals are reintroduced to the same task following a period of not being tested. In addition to ‘spontaneous recover’, extinction is also associated with ‘reinstatement’ and ‘renewal’. ‘Reinstatement’ refers to the re-appearance of a CR in animals exposed to the US between extinction and retention testing. ‘Renewal’ refers to the tendency of a CR to reappear in animals that have undergone extinction when retested in a different environment (Myers & Davis, 2002). Based on the gradual decline in contralateral accuracy, which was similar to what occurred when physically removing the sucrose pellet reward

following a correct contralateral response (Dowd & Dunnett, 2007), and the ‘spontaneous recovery’ like re-emergence of responding that was observed when lesion rats were retested after a test-free interval, Dowd and Dunnett (2007) hypothesised that the contralateral accuracy deficit observed in lesion rats represented extinction. This was hypothesised to be driven by an ablation of the dopaminergic reward signal that otherwise occur following presentation of a US (Schultz, 2010) in the lesion rats.

The LRCT task data reviewed above demonstrate the presence of motor deficits in the form of increased reaction and movement times following unilateral 6-OHDA lesions, as well as non-motor deficits that may reflect extinction (Carli *et al*, 1985, 1989; Dowd & Dunnett, 2004, 2007). The LRCT task may thus be used to measure deficits to both motor and non-motor function. This made it an appropriate task when conducting experiments designed to dissociate between the effect of dopaminergic drugs on motor and non-motor behaviour in rats with unilateral 6-OHDA lesions as part of this thesis project.

1.7. Pharmaceutical treatments for Parkinson’s Disease

The following section will provide an overview of pharmacological treatments for PD, and some common side effects associated with them before explaining the relevance of these side effects for the overall hypothesis the thesis project tested.

1.7.1. Anti-cholinergic drugs

The first effective pharmaceutical treatments for PD were anticholinergic drugs, which were introduced in the late 19th century. These anticholinergic compounds were extracted from the plant *Atropa Belladonna*, until the 1950’s, when synthetic compounds became available. Unfortunately, whilst long being the only pharmacological therapy showing clinical effectiveness, anticholinergic drugs offer limited relief when given as a monotherapy and are associated with severe side effects, including impaired psychosis, poor concentration, and memory disruptions (Katzenschlager *et al*, 2003; Smith *et al*, 2012; Sawada *et al*, 2013).

1.7.2. L-DOPA

Today, the most commonly prescribed pharmacological treatment for PD is the dopamine precursor L-DOPA. Being unable to cross the blood-brain barrier by passive diffusion, L-DOPA requires active transport via the LAT1 transport system (reviewed in del Amo *et al*, 2008). Once in the brain it is converted into dopamine by neurons containing the enzyme DOPA decarboxylase (Figure 1.5; Flatmark *et al*, 2000). L-DOPA is predominantly taken up by dopaminergic neurons where it is converted into dopamine and released into the synaptic

cleft, thereby temporarily restoring extracellular dopamine levels. In addition, it is known that some L-DOPA is taken up and converted into dopamine by serotonergic neurons which also contain DOPA decarboxylase, and other yet unidentified sources (Tanaka *et al*, 1999; Flatmark *et al*, 2000; Cenci & Lundblad, 2006; Lindgren *et al*, 2010).

L-DOPA's anti-Parkinsonian effects were first discovered in the late 1950's when the now Nobel laureate Arvid Carlsson showed that it reversed the akinetic effects of reserpine, a compound which blocks the vesicular monoamine transporter responsible for transporting cytoplasmic dopamine to presynaptic terminals (Carlsson *et al*, 1957). Based on Carlsson and colleagues' (1957) work, Birkmayer and Hornykiewicz (1961) administered L-DOPA to PD patients where it was shown to offer dose-dependent improvements of motor symptoms, and enable patients who had previously been restricted to sitting or lying positions to yet again stand and walk by their own accord (Birkmayer & Hornykiewicz, 1961, 1998). In line with their initial findings, acute administration of L-DOPA is now known to relieve the akinesia, bradykinesia, rigidity, and to a lesser extent also the tremor associated with PD (Birkmayer & Hornykiewicz, 1998; Miyasaki *et al*, 2002). While being superior to other pharmacological treatments in alleviating the motor symptoms of PD, L-DOPA is however associated with the onset of motor complications as further described in section 1.8.3 which limits its use.

1.7.3. Dopamine Agonists

Dopamine agonists act on postsynaptic receptors, where they mimic the actions of the dopamine that is lost in PD. Dopamine receptors are divided into D1-like (D1 and D5 receptors) and D2-like (D2, D3, and D4 receptors) receptors (Jaber *et al*, 1996). PD patients are not prescribed D1 agonists as these are implicated in the development of LID (e.g. Mela *et al*, 2012), but are instead prescribed D2 type agonists. Dopamine agonists are used either as a monotherapy or in conjunction with L-DOPA, which allows medical practitioners to reduce the dose of the latter. While D2 agonists are less effective than L-DOPA in treating the motor symptoms of PD they hold the advantage of inducing no, or only mild, LID if administered as a first-line treatment (Pearce *et al*, 1998). Agonist treatment is therefore commonly recommended in early PD (National Institute for Health and Clinical Excellence, 2006). Furthermore, late-stage PD patients have been shown to benefit from continuous infusion of the non-selective agonist apomorphine, as continuous delivery of dopaminergic medication instead of short-acting doses of L-DOPA is able to improve motor function while

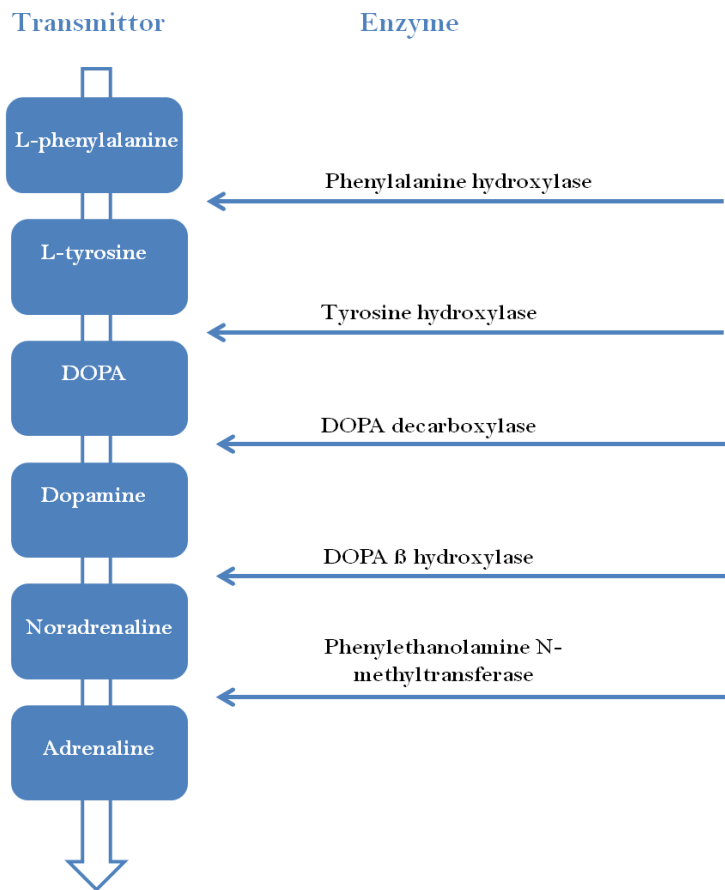


Figure 1.5. A representation of the synthesis and relationship between L-DOPA, dopamine, and their derivatives. Neurotransmitters and their precursors are shown in the blue boxes. The enzymes responsible for their conversion are listed above the arrows, which show the point at which each enzyme is involved in the synthesis. Adapted from Flatmark *et al* (2000).

also increasing the therapeutic window of dopaminergic medication and reducing dyskinesias (Garcia Ruiz *et al*, 2008).

1.7.4. Enzyme inhibitors

In addition to dopaminergic drugs, common PD treatments also include the administration of catechol-o-methyl transferase (COMT) and MAO enzyme inhibitors.

COMT inhibitors prevent the metabolism of L-DOPA, and thereby increase the bioavailability of L-DOPA in the brain. Co-administering COMT inhibitors with L-DOPA reduces motor fluctuations by increasing patients' "on time" and reducing their "off time" (1.8.3), offers symptomatic relief, and enables a reduction of patients' L-DOPA dose (Goetz *et al*, 2005; Talati *et al*, 2009; Deane *et al*, 2009; Stowe, 2011). However, despite preclinical data suggesting that COMT inhibitors can reduce dyskinesia in MPTP lesion monkey by

allowing a reduction of the L-DOPA dose necessary to achieve therapeutic effect (Huot *et al*, 2013), a meta-analysis of clinical trial data has suggested that COMT inhibitors administered as per current clinical practise may increase dyskinesias in patients (Talati *et al*, 2009). Based on available data, official UK prescription guidelines therefore recommend COMT inhibitors for reduction of motor fluctuations in late stage PD but not for purpose of reducing LID (National Institute for Health and Clinical Excellence, 2006).

MAO-inhibitors prevent the degradation of extracellular dopamine and therefore improve motor symptoms by prolonging the time that dopamine can act on post-synaptic receptors (Rascol *et al*, 2005). In the UK, official guidelines recommend MAO-B inhibitors as a monotherapy to achieve symptomatic relief in early PD, or in conjunction with L-DOPA in late PD (National Institute for Health and Clinical Excellence, 2006). MAO-inhibitors have been shown to increase the quality of patients' motor function as assessed by the Unified Parkinson's Disease Rating Scale, prolong the alleviating effects of L-DOPA on PD motor symptoms, and allow a 30-40% reduction in patients' L-DOPA dose (Gerlach *et al*, 1996; Rascol *et al*, 2005; Stowe, 2011). While MAO inhibitors improve motor side effects by reducing the daily time patients spend in "off" periods, their impact on dyskinesia is still under debate. Whilst single studies have reported that MAO-B inhibitors increase the time patients can spend on L-DOPA without experiencing disabling dyskinesias (Waters *et al*, 2004; Rascol *et al*, 2005), other studies have reported an increase in dyskinesias when co-administering MAO-B inhibitors with L-DOPA (Talati *et al*, 2009). Based on the available data, the most recent official UK guidelines recommend MAO-B inhibitors for the control of L-DOPA induced motor fluctuations but not for dyskinesia (National Institute for Health and Clinical Excellence, 2006).

In addition to the positive effects of MAO inhibition on PD motor symptoms, one commonly used MAO-B inhibitor, selegiline, has also been suggested to have a neuroprotective function. It is believed that this effect is due to inhibition of MAO-B mediated dopamine degradation, which reduces the formation of toxic compounds that would otherwise damage dopaminergic neurons (Gerlach *et al*, 1996). However, based on currently available clinical data, current official UK guidelines do not recommend administration of MAO-B inhibitors to PD patients for neuroprotective purposes unless tested as part of a clinical trial (Gerlach *et al*, 1996; National Institute for Health and Clinical Excellence, 2006).

1.8. Side effects of dopaminergic medication

1.8.1. Non-motor effects of acute L-DOPA administration

Administration of acute L-DOPA or dopamine agonists improve motor function in PD patients and animal models of PD (Birkmayer & Hornykiewicz, 1998; Miyasaki *et al*, 2002; Pearce *et al*, 1998; Stern, 2000; Miyasaki *et al*, 2002). However, in addition to relieving motor deficits these drugs also affect non-motor function.

For example, in primate MPTP lesion primates, L-DOPA doses that alleviate motor symptoms have been demonstrated to also impair performance on a delayed response task with high attentional demands (Schneider *et al*, 2009, 2013). Interestingly, the impaired performance on this delayed response task was ameliorated by co-administering nicotine, which increases dopamine clearance via its interaction with the dopamine transporter. The data have therefore been taken to suggest that increased cortical dopamine levels, as occurring following acute L-DOPA administration, disrupt attention (Decamp *et al*, 2009).

Acute L-DOPA has also been shown to improve PD patients' accuracy on task-switching and probabilistic learning tasks when patients are required to learn from positive feedback, while simultaneously impairing accuracy when patients' are required to learn from negative feedback (Cools *et al*, 2003; Frank *et al*, 2004). It has been suggested that acute L-DOPA administration mediates learning from positive feedback because the elevated dopamine levels that result from acute administration resemble the phasic dopamine signal that is typically observed following delivery of a US (Frank *et al*, 2004; Cools, 2006; Schultz, 2010). Simultaneously, the increase in dopamine levels following acute L-DOPA has been hypothesised to mask the depression in dopamine activity that typically follows the absence of an expected reward – thereby impairing learning that would otherwise result from absence of positive reinforcement (Frank *et al*, 2004; Cools, 2006; Schultz, 2010). Data from fMRI studies support the notion that acute L-DOPA affects the dopaminergic circuits implicated in feedback based learning. While healthy participants show activation in the cortico-striatal loop (the prefrontal cortex, caudate, thalamus, and the supplementary motor area) during positive feed-back mediated set-shifting, this activation is absent in PD patients tested off L-DOPA and partly restored following acute L-DOPA administration (Au *et al*, 2012).

Acute L-DOPA also affects impulsive behaviour such as gambling. This has been demonstrated by, for example, Cools and colleagues (2003) using a gambling task where the probabilities of winning could be calculated by the participant before placing a bet. Whilst L-DOPA did not affect the accuracy with which patients placed their bets, patients on

L-DOPA placed larger bets than patients tested off L-DOPA (Cools *et al*, 2003). The impulsive betting strategies are believed to be mediated by the ventral striatum which is less affected by the dopamine denervation observed in PD than the dorsal striatum (Cools *et al*, 2003). It is believed that L-DOPA doses that are able to offer relief of motor symptoms (reliant on dorsal striatal dopamine levels) simultaneously over-stimulate the less affected ventral striatum, and thereby enhance behaviours mediated by the ventral striatum such as impulsive behaviour (Cools *et al*, 2006).

1.8.2. Non-motor effects of acute dopamine agonist administration

Non-motor side effects have also been observed following dopamine agonist treatment. D2 agonists have been implicated in the development of impulse control disorders (ICDs), which one large cohort study estimated to affect approximately 14% of PD patients (Weintraub *et al*, 2010). ICDs are defined in the American Psychiatric Association's *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV) as being characterised by an inability, or severe difficulty, in resisting impulses that will result in negative consequences. ICDs associated with dopamine agonist use in PD include pathological gambling, hypersexuality, and dopamine dysregulation syndrome (Weintraub *et al*, 2010; Voon & Fox, 2007). These ICDs have been linked to the fronto-striatal and limbic-striatal circuitries (Voon & Fox, 2007) where, for example, there is increased release of ventral striatal dopamine when PD patients with ICDs engage in gambling (Steeves *et al*, 2009). Interestingly, while activity in healthy controls' ventral striatum shows stronger correlation with activity in the prefrontal cortex than in the insular during placebo conditions, acute D2/3 agonist administration causes the ventral striatum to instead show stronger correlation with activity in the insula. This has led to the suggestion that D2/3 agonists decrease top-down control over impulsive behaviour (mediated by prefrontal cortex) in favour of increased emotional influence on decision making (mediated by the insula) (Ye *et al*, 2011). On a behavioural level this has been suggested to cause overestimation of potential reward and an underestimation of potential risk, resulting in ICDs in a subset of PD patients (Camara, 2009).

1.8.3. Motor complications following chronic L-DOPA

L-DOPA's ability to offer greater symptomatic relief of PD motor symptoms than other pharmacological treatments for PD has made it the gold standard treatment in PD (Miyasaki *et al*, 2002). Unfortunately, the beneficial effects of L-DOPA on motor function are compromised by the development of adverse motor side effects following long-term use. The most notable side effect is LID, which affects approximately half of all patients after 5 years

of L-DOPA treatment (Nutt, 2001). L-DOPA induced dyskinesias are abnormal involuntary movements exhibited when patients are on L-DOPA treatment and consist of choreas and dystonia. The former are abrupt, hyperkinetic, movements of body limbs, whereas dystonia refers to slow, twisting, cramp-like movements of limbs (Cenci, 2007; Deane *et al*, 2009; Khan, 2012). Currently, the only clinically available treatment for the reduction of LID is the NMDA antagonist Amantadine (National Institute for Health and Clinical Excellence, 2006). Co-administering Amantadine with L-DOPA has been shown to reduce dyskinesia scores by approximately 60% without impacting on L-DOPA's beneficial effect on motor function (Verhagen *et al*, 1998).

In addition to LID, long-term L-DOPA treatment is associated with an increase in the duration of patients' so called "off" periods. These are periods during which the last L-DOPA dose has lost its effect but the next has not yet become effective. Hence, patients experiencing an "off period" no longer benefit from the drug's therapeutic effects and instead transiently return to an akinetic state (Marsden & Parkes, 1977; Khan, 2012).

1.8.4 Receptor changes following LID onset

Following initial dopamine loss, there is a sensitisation of D1-type receptors, and an upregulation of D2-type receptors, mGlu5, and NMDA receptors (Aubert *et al*, 2005; Sanchez-Pernaute *et al*, 2008; Ouattara *et al*, 2011; Wullner *et al*, 1994; Marti *et al*, 1999; Turjanski *et al* 1997; Ulas *et al*, 1994). LID onset further affects striatal receptors by increasing the binding to D1 receptors in MPTP lesion monkeys, whereas monkeys that remain non-dyskinetic following L-DOPA treatment show a normalisation of D1 binding (Table 1.1; Aubert *et al*, 2005). LID onset also enhances the upregulation of striatal mGlu5 receptors that occurs in MPTP lesion monkeys and PD patients (Sanchez-Pernaute *et al*, 2008; Ouattara *et al*, 2011). Furthermore, LID affects NMDA receptors, with PET imaging studies showing greater striatal NMDA receptor activity in dyskinetic than in non-dyskinetic patients when the patient cohorts were tested on, but not off, acute L-DOPA (Ahmed *et al*, 2011).

Table 1.1. An overview of changes to striatal receptors occurring following dopamine denervation and subsequent L-DOPA treatment, in dyskinetic and non-dyskinetic animals and patients. The table also shows whether the change was observed in patients, non-human primates, or rodents. If data was obtained using a preclinical model, the toxin used to mimick the dopamine loss observed in PD is indicated. References are displayed below the table.

| | Dopamine loss | Chronic L-DOPA | |
|--------------|---|--|--|
| | | Dyskinetic | Non-dyskinetic |
| D1 | Sensitised (MPTP primates ¹) | Further sensitization (MPTP primates ¹) | Normalised (MPTP primates ¹) |
| D2 | Upregulated (MPTP primates ¹) Increased binding (Patients ²) | No change in receptors (MPTP primates ¹ , patients ²) | No change (Patients ²) |
| mGlu5 | Upregulated (MPTP primates ³) | Further upregulation (Patients and MPTP primates ⁴) | Less binding than in dyskinetic animals/patients (Patients and MPTP primates ⁴) |
| NMDA | Sensitised & upregulated (6-OHDA rat ^{5,6}) Increased NMDA binding (Patients ⁷) | Increased binding relative to non-dyskinetic patients when on L-DOPA (Patients ⁸) | |

1: Aubert *et al* (2005); 2: Turjanski *et al* (1997); 3: Sanchez-Pernaute *et al* (2008); 4: Ouattara *et al* (2011); 5: Wullner *et al* (1994); 6: Marti *et al* (1998); 7: Ulas *et al* (1994); 8: Ahmed *et al* (2011)

1.8.5. Changes in synaptic plasticity following chronic L-DOPA

In addition to receptor level changes, LID has also been linked to changes in synaptic plasticity. Synaptic plasticity includes the three mechanisms long-term potentiation (LTP), long-term depression (LTD), and depotentiation.

LTP refers to the long-lasting potentiation of the synaptic strength between two neurons as the result of a previous short, but intense, synaptic activation and agonism of specific receptors (Bliss, 2003). LTP has been induced in cortico-striatal fibers *in vitro* by applying high frequency stimulation (HFS) whilst activating NMDA receptors by removing magnesium from the bathing solution the tissue is kept in (Calabresi *et al*, 1992, 2007; Centonze *et al*, 2003; Picconi *et al*, 2003). The success of the LTP induction can be

demonstrated by recording the excitatory post-synaptic potentials (EPSPs) elicited in the post-synaptic neuron following later application of HFS stimulation. The magnitude of EPSPs is greater post- than pre-LTP induction (Picconi *et al*, 2003).

LTD refers to a long lasting decrease in the synaptic strength between two neurons. LTD has been induced in cortico-striatal fibers *in vitro* by applying HFS to the tissue while NMDA receptors are inactivated by adding external magnesium to the bathing solution the tissue is kept in (Centonze *et al*, 2003). Like LTP, the presence of LTD may be measured by recording EPSPs or field potentials. Following LTD induction these are of lower magnitude than before induction (Bliss *et al*, 2003; Pisani *et al*, 2005; Calabresi *et al*, 1992a,b).

Following successful striatal LTP induction in cortico-striatal fibres, the potentiated response can be reversed *in vitro* by applying LFS to the presynaptic neuron. This process is known as depotentiation. Unlike LTD it does not represent a shift away from the baseline synaptic strength, but instead a reversal of the LTP induced potentiation (Picconi *et al*, 1992; Bliss *et al*, 2003; Pisani *et al*, 2005; Huang *et al*, 2010).

In vitro experiments have enabled a greater understanding of the induction of cortico-striatal LTP and LTD. Both forms of plasticity are blocked in cortico-striatal tissue from rats that have undergone 6-OHDA lesions (Calabresi *et al*, 1992a; Kerr & Wickens, 2001), suggesting a role of dopamine in both forms of plasticity. By using pharmacological blockade paradigms further insight into the receptors implicated in plasticity has been gained. Cortico-striatal LTP has been shown to depend on co-activation of mGlu1 & mGlu5 receptors (Gubellini *et al*, 2003), NMDA receptors (Calabresi *et al*, 1992b), and D1 or D5 receptors (

Table 1.2; Kerr & Wickens, 2001). Cortico-striatal LTD formation, on the other hand, has been shown to be dependent on activation of mGlu1 receptors, D5 receptors, and D2-type receptors (

Table 1.2; Calabresi *et al*, 1992a; Gubellini *et al*, 2001; Centonze *et al*, 2003). Cortico-striatal depotentiation has been induced *in vitro* in the absence of magnesium (Picconi *et al*, 2003) which suggests that its induction involves NMDA activation.

Furthermore, depotentiation has also been shown to require activation of protein phosphates 1 and 2A, and to be inhibited by D1 agonism (Picconi *et al*, 2003).

While cortico-striatal LTP and LTD are typically blocked in tissue from rats that have received 6-OHDA lesions (Calabresi *et al*, 1992a; Kerr & Wickens, 2001), *in vitro* data have demonstrated restored ability for LTP formation in tissue from lesion rats that were chronically treated with L-DOPA prior to culling (Picconi *et al*, 2003). Interestingly, the data demonstrated a difference in the ability for cortico-striatal plasticity in tissue from lesion rats

that did and did not develop LID in response to chronic L-DOPA treatment. Specifically, depotentiation of LTP could be induced in cortico-striatal tissue from L-DOPA treated non-dyskinetic rats, but not in tissue from rats that developed LID in response to the L-DOPA treatment (Picconi *et al*, 2003). This is believed to be due to the increased phosphorylation of

Table 1.2. An overview of the receptors that have been implicated in the induction of cortico-striatal long-term potentiation (LTP) and long-term depression (LTD).

| | mGlu1 | mGlu5 | NMDA | D1 | D5 | D2 type |
|------------|-------|-------|------|----|----|---------|
| LTP | x | x | x | x | x | |
| LTD | x | | | | x | X |

DARPP-32 that is associated with LID (e.g. Santini *et al*, 2007). Data obtained from sham lesion rats have shown that phosphorylation of DARPP-32, which is induced by D1 receptor activation, inhibits phosphatase 1 which is required for cortico-striatal depotentiation to occur (Picconi *et al*, 2003). Hence, the phosphorylation of DARPP32 that is known to occur in LID may underlie the loss of cortico-striatal depotentiation observed in tissue from lesion rats that have developed LID (Picconi *et al*, 2003).

Clinical data suggest that the disrupted synaptic plasticity observed in preclinical models may translate to the PD patients. In the clinic, LTP like plasticity may be induced in the motor cortex using protocols involving transcranial magnetic stimulation (TMS). One such protocol is the paired associative stimulation (PAS) method in which TMS applied over the motor cortex is paired with median nerve stimulation at the wrist. Changes in the amplitude of motor evoked potentials (MEPs) at the abductor pollicis brevis is then used as an indicator of potentiated responses. Using this protocol, Morgante and colleagues (2006) demonstrated a loss of LTP like plasticity in Parkinsonian patients tested off-medication. This plasticity was restored by acute administration of dopaminergic medication in non-dyskinetic, but not in dyskinetic, patients (Morgante *et al*, 2006). In line with preclinical findings (e.g. Picconi *et al*, 2003) the data thus demonstrated a requirement for dopamine for LTP like plasticity to occur in the cortico-striatal circuitry. However, the findings reported by Morgante and colleagues (2006) differed from preclinical data (Picconi *et al*, 2003) by being unable to induce LTP-like plasticity in dyskinetic participants. One possible reason for the discrepancy is the protocols used: whereas preclinical experiments (e.g. Picconi *et al*, 2003) study LTP directly in cortico-striatal tissue, Morgante and colleagues' (2006) protocol only measured

LTP indirectly by measuring the effects of TMS on motor MEPs. There was thus a difference both in the methodology used and the specific brain structures being studied.

A later study readdressed the issue using a new protocol that had been demonstrated to be more consistent in its ability to induce LTP like effects in PD patients (Huang *et al*, 2010, 2011). With this protocol, it was also possible to study both LTP like and depotentiation like plasticity (Huang *et al*, 2010, Huang *et al*, 2011). In their study, Huang and colleagues (2011) were able to induce LTP like potentiation in both dyskinetic and non-dyskinetic patients when these received their full L-DOPA dose. Furthermore, in line with preclinical data (Picconi *et al*, 2003) only the non-dyskinetic group was found to have retained the ability for depotentiation. The data from Huang and colleagues (2011) therefore suggested that preclinical findings of disrupted synaptic plasticity in a model of PD (Picconi *et al*, 2003) may translate to the clinic. The data further suggested that the failure of an earlier study (Morgante *et al*, 2006) to demonstrate LTP like plasticity in dyskinetic PD patients may have been due to the methodology used.

The hypothesis that subtle differences in methodology and patients medication have a large impact on the ability to induce LTP like plasticity using PAS protocol is supported by data from other clinical experiments. In the previously described study, Huang and colleagues (2011) were able to induce LTP like plasticity in PD patients receiving their full L-DOPA dose. However, they failed to induce LTP like plasticity when patients received half their dose (Huang *et al*, 2011). This is consistent with data from other studies where LTP-like plasticity could not be induced in patients receiving a low L-DOPA dose prior to testing (Suppa *et al*, 2011). Together, these studies show that the ability for inducing LTP like potentiation in patients is highly sensitive to not only the protocol (Morgante *et al*, 2006, Hunag *et al*, 2010, 2011) but also the dose of medication the participants receive (Suppa *et al*, 2011, Huang *et al*, 2011).

A weakness of the clinical studies described above (Morgante *et al*, 2006; Huang *et al*, 2010, 2011; Suppa *et al*, 2011) is that they only measured LTP and depotentiation indirectly - unlike *in vitro* studies which are able to directly measure the effect of potentiation on post-synaptic responses. One instance in which synaptic plasticity can be measured directly in the clinic is during deep-brain stimulation (DBS) implantation, a surgical therapy aimed to relieve PD symptoms by implantation of electrodes to the basal ganglia (Starr *et al*, 1998). During such implantation, Prescott and colleagues (2009) were able to record field evoked potentials (fEP) following HFS in the SNr of patients that were either on or off L-DOPA medication at the time of surgery. Prescott and colleagues (2009) demonstrated that

LTP could only be induced in patients who were on L-DOPA at the time of testing. Whilst recording from the SNr (Prescott *et al*, 2009) rather than cortico-striatal tissue as in the previously described preclinical study (Picconi *et al*, 2003), the data offer further support for findings of a disruptive effect of PD on basal ganglia plasticity, and the need for dopamine for successful LTP induction in the basal ganglia network, as well as suggesting that the abnormal plasticity associated with PD may affect multiple parts of the basal ganglia circuitry.

Whilst further work is required to understand the precise effect of PD and acute and chronic L-DOPA on basal ganglia synaptic plasticity, the cited studies suggest a long-term effect on synaptic plasticity on basal ganglia plasticity. These changes, together with the receptor changes also associated with L-DOPA and LID onset, could both be hypothesised to affect non-motor behaviour mediated by the striatum. In addition to motor function, the striatum is known to mediate non-motor functions such as motor learning, and goal-directed, and habitual behaviour (e.g. Wächter *et al*, 2010; Yin *et al*, 2004, 2005; Reynolds *et al*, 2001; Luft *et al*, 2004; Okulski *et al*, 2002). Therefore, further to the already known effect of chronic L-DOPA on the development of LID and motor fluctuations (Marsden & Parkes, 1977), chronic exposure to the drug may impact on non-motor functions.

1.9 Thesis aims

In the 1960's, the reported link between dopamine loss and akinesia led to the dopamine precursor L-DOPA being introduced as a pharmacological treatment for PD motor symptoms (Ehringer & Hornykiewicz, 1960; Birkmayer & Hornykiewicz, 1961). The therapeutic relief provided by L-DOPA made it the gold standard treatment for PD, However, it is now recognised that L-DOPA affects a wider range of behaviours than initially realised. Acute L-DOPA administration is known to affect both motor function (Birkmayer & Hornykiewicz, 1961) and cognition (e.g. Cools, 2006). Conversely, while long-term L-DOPA is known to induce motor side effects (Marsden & Parkes, 1977), little is known about the effects of long-term L-DOPA treatment on non-motor function. This is in spite of data showing that chronic L-DOPA exposure and LID onset are associated with receptor and plasticity changes to the striatal system (e.g. Aubert *et al*, 2005; Picconi *et al*, 2003; Prescott *et al*, 2009; Huang *et al*, 2011), and knowledge that this system is implicated in non-motor functions such as motor learning, goal-directed behaviour, and habitual behaviour (Wächter *et al*, 2010; Yin *et al*, 2004, 2005). The purpose of this thesis was to address the gap in the current scientific

understanding by exploring whether the effects of long-term L-DOPA exposure and LID onset extended beyond motor side, to also affect non-motor behaviour.

The main aims of the thesis were:

- To determine whether the chronic L-DOPA impairs motor learning and non-motor function in the 6-OHDA rat model of PD.
- To compare the effect of chronic L-DOPA and a D2 agonist, which does not induce LID, on motor learning and non-motor function in the 6-OHDA rat model of PD.
- To, once a deficit had been identified, dissociate the effects of chronic L-DOPA and the development of LID on said deficit.

2. Methods

2.1 Rats

2.1.1 Animal husbandry

Experiments were conducted in the School of Biosciences, Cardiff University, with the exception of Experiment 5 which was conducted at Eli Lilly & Co Ltd, Windlesham, Surrey, UK. For this reason, there were minor differences in the animal husbandry in Experiment 5 relative to the other experiments, as specified below.

Rats were housed in groups of 1-4 rats per cage in all experiments apart from in Experiment 5, where they were housed in groups of 1-5. The holding room light was set to a 14:10 h light/dark cycle in all experiments apart from Experiment 5, in which a 12:12 h light/dark cycle was used.

In accordance with Home Office regulations, the room temperature was kept between 19°C and 23°C and the humidity at 55% ± 10%. The cage floor was covered by sawdust, and basic enrichment provided in the form of a cardboard tunnel and wooden chewing sticks in all experiments apart from Experiment 5, where rats were given a plastic nest ball and wooden chewing sticks. Rats were given *ad libitum* access to water and, when not on food restriction, food chow. The diet consisted of Teklad Global 14% Protein Rodent Maintenance Diet (Harlan Laboratories, UK) in all experiments apart from that Experiment 5, where Tekland Global 16% Protein Rodent Maintenance Diet (Harlan Laboratories, UK) was used.

2.1.2. Rats used & suppliers

All Lister Hooded rats were obtained from Charles River (UK). Sprague Dawley rats were obtained from Harlan (UK), apart from the Sprague Dawley cohort included in Experiment 5 which was obtained from Charles River (UK).

2.1.3. Food restriction

Food restriction, when used in this thesis, refers to the practise of restricting rats' food intake from *ad libitum* to being fed once daily in such volumes that the rats' weights are maintained at between 85% and 90% of their baseline weight.

In instances where rats were food restricted prior to reaching adulthood their target weights were adjusted to allow for further growth. Based on growth curves provided by the animal suppliers, adulthood was considered reached when female rats weighed ≥ 250 g, and

male rats weighed ≥ 350 g. For rats that were not considered to have reached adulthood, the minimum target weight was set to 85% of baseline weight and the maximum target weight was set to 90% of baseline weight during their first week of food restriction. In subsequent weeks, the upper and lower target weights were increased by 5 g per week until the rats were fully grown.

2.2. Surgery

2.2.1. General surgery procedures

Rats that had previously been food restricted were given *ad libitum* access to food for a minimum of two days prior to surgery.

Prior to surgery, 1g 6-OHDA containing ascorbic acid (cat no H116, Sigma-Aldrich) was mixed with 0.8 ml 0.9% saline. This caused the final solution to have a free base concentration of 5.14 mg/ml. The 6-OHDA solution was pipetted into eight aliquots, containing 100 μ l solution each, immediately after the saline and 6-OHDA had been mixed. The aliquots were wrapped in tin foil to protect them from light and stored at -20°C . On the day of surgery, one aliquot at the time was taken to surgery where it was kept in an ice filled polystyrene box, with the lid kept on at all times to prevent exposure to light. The colour of the toxin was closely monitored and an aliquot was discarded and replaced as soon as the 6-OHDA started turning pink.

Rats were anaesthetised in induction chambers using 2-4% isoflurane with NO_2 and O_2 simultaneously administered at a 2:1 ratio. Once unconscious and no longer showing a response to pinching of the tail tip or hindlimb digit the rats were moved to stereotaxic frames (Kopf) where anaesthesia was maintained using the same isoflurane, NO_2 and O_2 concentrations as during induction. Blunt ear bars were used to stabilise the head. An incision was made across the skull above bregma using a sharp scalpel. The exposed skull surface was thereafter cleared using the scalpel blade and cotton buds. Forceps were used to prevent the skin from closing the wound during surgery.

Lesion coordinates were calculated from bregma, which was identified using a surgery microscope. Once the coordinates had been identified, a small hole was drilled through the skull, and 6-OHDA was infused using a 30 gauge cannula. A syringe pump and a Hamilton syringe, connected to the cannula by polyethylene tubing, were used to infuse the toxin at a speed of 1 μ l/min. Following infusion and diffusion of the toxin, the rats received a 5 ml subcutaneous injection of 0.9% saline (AnimalCare, UK) to prevent dehydration and the

wound on the skull was stitched together using vicryl sutures. Rats were thereafter placed in heated recovery cages until they were awake and alert enough to be returned to the holding room. Once recovered from anaesthesia, rats were returned to clean cages, prepared as described previously (2.1.1) but including nesting material for warmth for the first two nights post surgery. Rats were checked daily for a minimum of three days, with special attention paid to their weights, the healing of the wound, and alertness.

Because of changes in the standard practises in the School of Biosciences, Cardiff University, and because one of the experiments (Experiment 5) was conducted in a different location (Eli Lilly & Co Ltd, Windlesham, Surrey) and under a different project licence there were differences in the post surgery analgesics used in different experiments. In the experiments described in Chapters 3 and 4, analgesic was provided in the form of paracetamol dissolved in their drinking water upon return to the home cages. Paracetamol was administered for two days post-surgery. In the experiment described in chapter 5 analgesic was provided by a s.c. injection of 0.15 ml buprenorphine (Vetergesics, UK) administered after infusion of 6-OHDA. In the experiments described in Chapters 6-8, analgesic was provided by a s.c. injection of 30µl Metacam (Boehringer Ingelheim, UK) after infusion of 6-OHDA.

2.2.2. Medial forebrain bundle lesions

Lesions to the MFB were conducted by infusing 6-OHDA into AP -4.0, ML -1.3 from bregma, and DV -7.0 below dura mater. The tooth bar was set to -4.5mm. The toxin was infused for 3 min at a rate of 1 µl/min, causing 15.42 µg of freebase 6-OHDA to be infused. The cannula was left in place a further 2 min before removal, to allow for diffusion around the infusion site.

2.2.3. Intra-striatal lesions

Intra-striatal lesions were conducted by infusion of 6-OHDA across eight sites. The tooth bar was set to -4.5mm. Four holes were drilled at AP +1.0, ML-2.0; AP +0.4, ML -2.8; AP -0.2, ML -3.6; and AP -0.8, ML -4.4 relative to bregma. Infusion took place at two depths. First, the cannula was lowered to DV -5.5 below dura mater and 6-OHDA was infused for 1 min. The cannula was then retracted to DV -4.5 below dura mater and 6-OHDA was infused for 1 more min at a speed of 1 µl/min. The cannula was thereafter left at DV-4.5 for 2 min to allow for diffusion around at the infusion site before retracting the cannula.

2.3. Drug preparation & administration

2.3.1. Bromocriptine

Bromocriptine was stored in a 4°C fridge. During preparation, it was mixed with an equal weight of tartaric acid and placed in a sonicator where 1-2 drops of ethanol was added. Once the solution had dissolved, distilled water was added to create the desired concentration. The drug was administered i.p. at a volume of 2 ml/kg.

2.3.2. L-DOPA

L-DOPA was stored in a -20°C freezer. During drug preparation, benserazide hydrochloride was added to the weighed out L-DOPA and both drugs were dissolved in 0.9% saline. Benserazide hydrochloride was always administered at a concentration of 15 mg/kg. The L-DOPA solution was prepared immediately before administration and injected at a volume of 1 ml/kg. L-DOPA was always administered via s.c. injections to the scruff, as s.c. administration has been demonstrated to produce less variable plasma levels of the drug than i.p. injections (Lindgren *et al*, 2007). When administered on a daily basis, care was taken to alternate the site of injection between the right, left, and central scruff to avoid soreness.

2.3.3. Met-amphetamine

Met-amphetamine was stored in room temperature. The drug was dissolved in 0.9% saline immediately before administration and injected i.p. at a concentration of 2.5 mg/kg and a volume of 1 ml/kg.

2.3.4. Raclopride tartrate

Raclopride tartrate was weighed out and dissolved in sterile 0.9% saline immediately before injection. The drug was administered i.p. at a volume of 1 ml/kg.

2.4. Motor behavioural tests

2.4.1. The 100 pellets test

Food restricted rats were placed in empty transparent activity cages (section 2.4.4). The bottom of the activity cages measured 20 cm wide and 36 cm long, whilst the top measured 26 cm wide and 41.5 cm long. The height was 18.5 cm. A metal grid cage lid was placed on top of the cage during testing to ensure the rats' remained inside. The lid was made of 30 grids running across the length of the cage, located 1 cm apart from each other, and 11 grids running across the width of the cage, located 4 cm apart from each other.

In the beginning of a testing session, disposable plastic drug weigh boats containing 100 sucrose pellets (AIN-76A Rodent Tablet, TestDiet, UK) were placed in the cage. Using a count-up timer, the time taken for rats to eat the available pellets was measured. There was no upper time limit and all tested rats consumed the available sucrose pellets.

2.4.2. Activity cages

Activity cages from TMed Associates Inc. (Vermont, USA) were made of transparent Plexiglas and positioned on a metal rack. On either side of the cage were three continuous laser beams going across the bottom of the cage. Perservative and non-perservative beam breaks were recorded and used as an indicator of rats' activity levels.

The bottom of the activity cages measured 20 cm wide and 36 cm long, whilst the top measured 26 cm wide and 41.5 cm long. The height was 18.5 cm. A metal grided cage lid was placed on top of the cage during testing. It consisted of 30 grids running across the length of the cage, 1 cm apart, and 11 grids running across the width of the cage, 4 cm apart.

2.4.3. Adjusting step test

The adjusting step test was first described by Olsson *et al* (1995) to measure forelimb akinesia in the hemi-Parkinsonian rat and is known to be sensitive to both dopamine denervation and anti-Parkinsonian drugs. The test was conducted in accordance with the previously published protocol (Olsson *et al*, 1995). Rats were restrained in such way that they could only use one forelimb, and thereafter moved along a 1 m flat surface while ensuring that the free forelimb was in constant contact with the surface. To ensure all rats were constrained at a consistent distance from the table top during testing they were held in such a way that the experimenters' knuckles were always in contact with the table top whilst restraining the rats.

Rats were moved in the forehand and backhand direction across the 1 m distance three times during testing of the ipsilateral and contralateral forelimb, respectively. The average number of adjusting steps the made using the paw ipsilateral and contralateral to the lesion was recorded and used as an indicator of forelimb akinesia.

2.4.4. The Cylinder Test

Background

The cylinder test was developed by Schallert and colleagues (2000) as a measurement of forelimb motor function in the laboratory rat. The cylinder test is a non-invasive test which draws on rats' inquisitive nature. It measures rats' use of their right and left forelimb whilst

exploring a novel cylinder environment. Whilst intact rats use both forelimbs to an equal extent, rats with unilateral 6-OHDA lesions or hemispinal damage preferentially use their unaffected forelimb when exploring the cylinder (Schallert *et al*, 2000). Dopaminergic drugs have previously been demonstrated to reduce the ipsilateral bias exhibited by rats with unilateral 6-OHDA lesions making it a useful tool in preclinical PD research (Lundblad *et al*, 2002).

Apparatus

The cylinder (in-house manufacturing) was made of transparent plexiglas and had diameter of 25 cm and a height of 43 cm. Two mirrors were placed behind the cylinder to allow observation of paw placements made whilst rats were turned away from the experimenter.

Testing

Each session was filmed using a 3.1 mega pixel Panasonic NV-GS330 cam recorder that was mounted on a tripod. Each session began when a rat was placed in the cylinder by the experimenter. The rat remained in the cylinder until the experimenter, who used a clicker to count the number of paw placements made, had observed a minimum of 15 paw placements. A paw placement was defined as a weight bearing placement of either forelimb against the wall of the cylinder. The first ten paw placements were used in the analysis, where they were recorded as being made by either the ipsilateral or contralateral paw. If it was unclear whether or not a paw placement had been made, a note was made by the experimenter and the video recording was used to confirm the number of ipsilateral and contralateral paw placements that had been executed.

2.4.5. Elevated body swing

The elevated body swing test has previously been described by Borlongan & Sanberg (1995) who demonstrated an ipsilateral bias in rats with unilateral 6-OHDA lesions, and a positive correlation between rats' ipsilateral bias on the elevated body swing test and the ipsilateral bias of their apomorphine induced rotations. Testing began with rats being picked up from a flat table surface by the base of their tails. Care was taken to ensure that the rat's trunk was not bent in either direction when they were picked up by the experimenter. Rats were then held approximately 20 cm above the table top surface until they lifted their bodies by more than 30° in either direction at which point the movement was recorded as being to either the ipsilateral or contralateral side of the lesion and the rat was returned to the surface. Rats were tested three times, with a short break in between each trial during which they were allowed to walk freely on the table top to ensure they remained calm during testing.

2.4.6. Rotometer testing

A schematic representation of the rotometers is provided in Figure 2.1. The rotometers and the control unit were both manufactured by Med Associates Inc, USA. Prior to commencing testing the rats were tethered using an elastic band, with the exception of Chapter 5 where plastic, self-locking cable ties were used. Rats were thereafter placed in cylindrical rotometer pots (dimensions specified below). The rats' harnesses (i.e. the elastic bands or cable ties) were connected to a suspended, elastic cord via a hook. The cord was attached to a mechanical rotation sensor which rotated when the rats did. Rotation of the sensor was recorded by the software. The settings were adjusted to record partial/ 90° turns per count, in 1 min time bins, with 8° retrace.

Rats were placed in bowls during testing. All experiments, apart from Experiment 5, were conducted at the School of Biosciences, Cardiff University where the bowls were made of transparent plastic and had an upper diameter of 30 cm, and a bottom diameter of 15 cm (Whitefurze, UK). The bowls were placed inside transparent, plastic cylinders that acted as walls and ensured that the rats' remained within the rotometer bowls. Fresh sawdust was placed in the bowl prior to each testing session. Experiment 5 was conducted at Eli Lilly & Co Ltd, Windlesham, Surrey, where different pots were used. These pots were made of metal and had a height of 45 cm and a diameter of 45.5 cm, and were surrounded by non-transparent plastic walls.

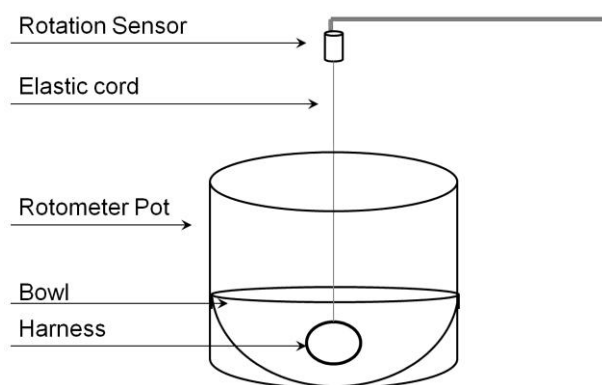


Figure 2.1. A schematic representation of an automated rotometer.

2.4.6.1. Amphetamine induced rotations

When developing the 6-OHDA rat model of PD, it was noted that unilateral lesions caused rats to spontaneously turn towards the side of the lesion (Ungerstedt, 1968). It was also shown that the ipsilateral bias in rats' locomotion could be enhanced following acute

administration of amphetamine, which causes release of dopamine from dopaminergic terminals whilst also preventing dopamine reuptake (Ungerstedt & Arbuthnott, 1970). Amphetamine induced rotations have since been used as a time-efficient method for screening lesion extent in following unilateral 6-OHDA lesions to the dopaminergic system.

When amphetamine induced rotations were used in this thesis project, rats were placed in the rotometers, their harnesses were attached, and the rats were then given an i.p injection of 2.5 mg/kg met-amphetamine at a volume of 1 ml/kg immediately before the recording started. Rotational behaviour was recorded for 90 min post-injection.

2.4.6.2. Spontaneous rotations

Recording of rats' spontaneous rotations was conducted after 7pm as rats are more active close to, and during, their dark cycle which began at 8pm in the experiments where spontaneous rotations were recorded. The light in the rotometers room was switched off throughout testing to increase the rats' activity levels. In each spontaneous rotation session, rats were placed in the rotometers one at the time and their rotational behaviour recorded for 10 min. To ensure maximal activity, no habituation period was provided. Instead, the software started recording immediately after each rat was placed in her rotometer pot.

2.4.7. The Staircase Test

Background

The staircase test was developed by Montoya and colleagues (1991) as a means for measuring fine motor skills. It takes place in so called staircase boxes where it measures food restricted rodents' ability to collect sucrose pellets using their ipsilateral and contralateral forelimb respectively. The retrieval of sucrose pellets requires successful advancement of the forelimb towards the pellet, pronation of the digits, grasping of the pellet by closing the digits around it, and finally moving the grasped pellet back to rat's head for consuming. Whilst intact rats readily collect sucrose pellets using both paws, unilateral 6-OHDA lesions impair rats' ability to retrieve pellets using the forelimb contralateral to the lesion (Montoya *et al*, 1991). The movement sequence involved in reaching for, and grasping an object has been shown to be highly similar between rats and people which adds to the clinical validity of the test (Sacrey *et al*, 2009).

Apparatus

A picture of a staircase box is shown in Figure 2.2. The staircase boxes consisted of three parts; a main box with a lid on top into which the rats were initially placed, an extension

containing a platform supporting the rats' bodies during testing, and a removable staircase that was inserted into the extension during testing. At the bottom of the main box was a metal grid, where grids placed 1cm apart ran across the width of the box. The lid on the main box and the extension were made of transparent Plexiglas, the platform and the staircase were both made of non-transparent plastic, and the walls of the main box were made of either non-transparent plastic or metal.

The main box was 11.5 cm wide, 20 cm long, and had a height of 20 cm. The extension was 16.5 cm long, 6 cm wide, and had a 6 cm gap between the platform and the ceiling. The steps on the staircase were 1.9 cm long, 1.5 cm wide and had an indentation in the middle into which sucrose pellets (AIN-76A Rodent Tablet, TestDiet, UK) were placed. The indentation was 0.5 cm deep and had a diameter of 1.7 cm. The staircase was 4.32 cm high, with the lowest step being located 0.5 cm above the ground.

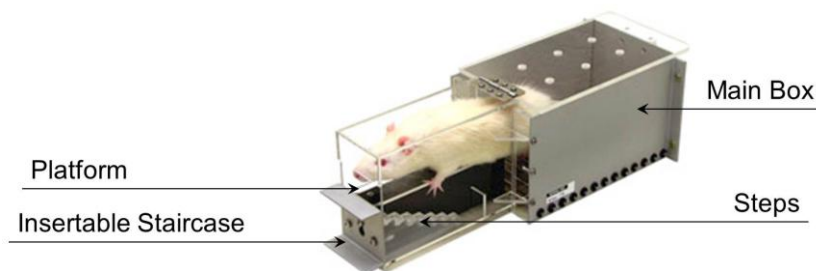


Figure 2.2. Picture of a rat staircase box. Picture modified from Campden Instrument's website.

Testing procedure

Prior to testing, a staircase baited with 45 mg sucrose pellets (AIN-76A Rodent Tablet, TestDiet, UK) was inserted into the staircase box. The number of pellets placed on each step was either 3 or 4, as detailed in the individual experiment methods sections. Following baiting of the staircase, food restricted rats were placed in the staircase box by the experimenter who thereafter did not interact with the rats until the end of the session. Rats were allowed 15 min in the staircase boxes after which they were returned to their home cages.

At the end of each testing session, the number of pellets remaining on each step was counted to provide a measurement of ipsilateral and contralateral forelimb use. In addition, the number of displaced pellets was counted as an indicator of attempted but failed retrievals.

A displaced pellet was defined as a pellet that was known to have been pushed from one step to a lower step. For example, if a step had been baited with 3 pellets, but 5 pellets were located on it at the end of staircase testing, two of these pellets must have been pushed there from an above step and were therefore defined as displaced. Based on the collected data, it was also possible to determine the furthest away step the rats had been able to access, which was used as an indicator of reaching ability.

2.4.8. The Vibrissae Test

The vibrissae test was first described by Woodlee *et al* (2005). It is used to measure sensorimotor reflex in the hemi-Parkinsonian rat and has been shown to be sensitive to both 6-ODHA lesions and antiparkinsonian drugs (Woodlee *et al*, 2005; Pinna *et al*, 2010).

During testing rats were restrained in such a way that they only had free use of one forelimb. Rats were held just below a bench top with their bodies parallel to the bench surface and then lifted in an upwards direction so that the whiskers on the same side of the body as the unrestricted forelimb gently brushed against the side of the table top. An intact rat responds to this stimulation by placing their corresponding paw on the bench top. Rats with unilateral 6-OHDA lesions, however, make fewer responses following stimulation of their contralateral whiskers than following stimulation of their ipsilateral whiskers.

A score of 1.0 was given if the rats responded to the stimulation by raising their unrestricted paw and placing it on the table top. A score of 0.5 was given if the rats responded to the stimulation by raising their unrestricted paw but failed to place it on the table top. The procedure was repeated ten times before turning the rat round and repeating on the other side.

2.5. Dyskinesia Scoring

Abnormal involuntary movements (AIMs) were defined as affecting the side of the body contralateral to the lesion, being atypical, and not serving any observable purpose. While the original AIMs scoring protocol (Cenci *et al*, 1998) rated four subtypes of AIMs (axial turning, locomotion, orolingual movements, and forelimb AIMs) on a scale from 0 to 4 depending on the time per scoring session the AIMs were present, the scoring protocol has since been modified to also include scoring of AIMs severity (Winkler *et al*, 2002). The refined scoring scale is as such more sensitive, and it has been demonstrated that scores obtained using the refined scale correlate significantly with molecular markers of LID (Winkler *et al*, 2002).

This thesis project scored AIMs using Winkler *et al*'s (2002) scale, as scoring both duration and severity of AIMs was considered to enhance the sensitivity of the scores. In addition, hindlimb dystonia which is considered to mimic the dystonia that forms part of human LID (Steece-Collier *et al*, 2003) and has also been reported by other researchers (Breger *et al*, 2013) was scored. Locomotor AIMs were not reported these have been demonstrated to not be reduced following administration of a clinically available anti-dyskinetic drug (Lundblad *et al*, 2002), which questions the validity of locomotor AIMs as a model for human LID.

As such, four AIMs subtypes were scored affecting the forelimbs, hindlimbs, axial angle, and orolingual function respectively. All AIMs were given both duration and severity scores. Duration scores ranged from 0 to 4 (Table 2.1). A score of 0 indicated that no AIM was observed; 1 that it was present < 50% of the time; 2 that it was present > 50% of the time but not continuous; 3 that the movement was continuous but could be interrupted by a sudden noise or movement; and 4 that the movement was continuous and could not be interrupted by sudden noises or movements. Severity scores ranged from 0-2 for orolingual AIMs, and 0-4 for all other AIMs as detailed in Table 2.2.

The rats' AIMs were scored for 1 min every 20 min for a total duration of 2 h following drug administration in Chapter 4, and for 3 h following drug administration in all other experiments. When calculating the overall score for each time point, the duration and severity scores for each subtype were multiplied and the products added together to produce a final, cumulative AIMs score. The maximum score a rat could obtain at one scoring point was 32. In a 2h scoring session the maximum achievable score was 192, and in a 3h scoring session the maximum achievable score was 288.

A rat was classified as non-dyskinetic if it never exhibited a severity score >1 on the axial, forelimb, or orolingual AIMs subtype at any scoring session. This cut-off has previously been shown to distinguish between rats with high and low striatal levels dyskinesia markers such as phosphorylated ERK1/2 (Westin *et al*, 2007). FosB, and PDyn mRNA (Andersson *et al*, 1999). In addition, following acute L-DOPA administration, rats classified as non-dyskinetic using this cut-off have lower levels of extracellular DOPA in the SNc and striatum than dyskinetic controls (Lindgren *et al*, 2010) and, unlike dyskinetic rats, do not show an angiogenic response to L-DOPA treatment (Westin *et al*, 2006).

Table 2.1. The scoring criteria used to determine duration for AIMS.

| 1 | 2 | 3 | 4 |
|--|--|--|---|
| The abnormal movement is present <50% of the time. | The abnormal movement is present >50% of the time. | The abnormal movement is continuous, but can be interrupted by sudden noises or movements. | The abnormal movement is continuous, and cannot be interrupted by sudden noises or movements. |

Table 2.2. The scoring criteria used to determine the severity of AIMS.

| | 1 | 2 | 3 | 4 |
|------------|---|--|--|---|
| Forelimb | Small, repetitive movements around a fixed position, usually on the ground. | Small movements, engaging both the distal and proximal forelimb. Usually close to the mouth/head. | Large movements engaging the whole forelimb and causing contraction of the shoulder. Usually occurring near the trunk. | Large, vigorous movements engaging the whole limb and shoulder. Usually occurring near the trunk. |
| Hindlimb | Foot angled away from the body, and pushed slightly forwards. | Foot angled away from the body. The whole hind leg tense and pushed forwards, often affecting balance. | | |
| Axial | The head and neck are maintained at a lateral deviation of approximately 30°. | The head and neck are maintained at a lateral deviation of approximately 30°-60°. | Lateral deviation also affecting the trunk, with a lateral deviation of 60°-90°. | The lateral deviation of the trunk, head, and neck are >90° and cause a loss of balance. |
| Orolingual | Chewing movements of the jaw. | Protrusions of the tongue. | | |

2.6. Microdialysis

2.6.1. Probe implantation

Prior to surgery polyethylene tubing was attached to each probe's inlet and outlet. Rats were anaesthetised and prepared for surgery as described in section 2.2.1.

The bilateral implantation sites were located AP ± 0.1 , ML ± 3.2 from bregma, and DV-5.0 from dura mater. Prior to inserting the probe, two screws were mounted caudally of the drill holes at such depths that they were securely attached to the skull without penetrating

it. A stereotaxic frame with two manipulator arms was used to lower the probes into the implantation sites.

During implantation, artificial cerebrospinal fluid (aCSF; 77.78% pure water with 10.10% 141 mM NaCl, 10.10% 5mM KCl, 1.01% 1.5mM CaCl₂, and 1.01% 0.8mM MgCl₂) was pumped through the probes' inlet tubes at a speed of 5 µl/min using a Hamilton syringe pump. In intact probes the aCSF flows unhindered through the probe and out via its outlet tube. Disrupted flow indicates damage to the probe membrane. Therefore, if flow became disrupted while the probe was lowered, the probe was retracted and replaced.

Once both probes were in the correct position and the experimenter had ensured a constant, unhindered flow of aCSF through the probes, dental cement was used to attach the probes to the screws and the skull. Care was taken to ensure the exposed skull was clean and dry prior to applying the dental cement. Once the dental cement had dried, the tubing connected to the probes' inlets and outlets were sealed using a soldering iron to prevent air and dust from entering the system. The rat was then given a s.c. injection of 0.15 ml buprenorphine (Vetergesics, UK) in the scruff and placed in a heated recovery chamber until fully recovered from surgery. Rats were monitored closely for a minimum of 2 h post implantation. They were thereafter housed individually and checked for a minimum of 3 times/day for 48 h following implantation.

The micodialysis probes were custom made from Microbiotech (Sweden) and had 7 mm guide shafts and 3 mm membranes. The membrane was a 6000 Dalton cut-off cuprophane membrane, stabilised with glycerol, and with an outer diameter of 0.24 mm.

2.6.2. Dialysis

Dialysis took place in a designated room where lights were kept dimmed throughout the dialysis session. Rats were weighed and then placed in cylindrical, non-transparent cylindrical pots that contained sawdust from their respective home cages. The pots were 30 cm tall and had a diameter of 35 cm. All rats were given a hydrogel (Harlan, UK) in their pots to prevent dehydration. *Ad libitum* rats also had food pellets placed in their pots.

During testing, rats wore a harness in the form of a plastic electrical tie loosely fitted beneath their forelimbs. The harness was attached to an elastic wire by a clip. The wire was suspended from a lever attached to the top of the pot via a rotatable swirl, which allowed the rats to move freely in the pot without disrupting the system.

Dialysis samples consisted of a D sample, a P sample, and 16 experimental samples. The D sample was collected prior to connecting the rat to the dialysis system, by running the

artificial CSF through the tubing system for 5 min using an automated Hamilton pump with the rate set to 5 μ l/min. After collecting the D sample, and prior to collecting the P sample, the soldered tip of the polyethylene tubes attached to each probe's inlet and outlet were cut with a scalpel and connected to two polyethylene tubes which ran via the inside of the elastic wire to the dialysis syringe pump and sample vial respectively. Following connection of the tubing, the P sample was collected over a 1 h period with the pump set to 2 μ l/min. The following 16 experimental samples were collected every 15 min, with the flow rate maintained at 2 μ l/min. Rats were administered a s.c. injection of 5 mg/kg L-DOPA, to the scruff at the beginning of the sixth sample.

2.6.3. HPLC

All HPLC was conducted by Jane Cooper at Eli Lilly & Co Ltd (Windlesham, Surrey) who also analysed the chromatograms and provided the author of this thesis with the numerical data.

The mobile phase was made up by 100 mM NaH₂PO₄, 1.6 mM OSA, and 14% MeOH with the pH set to 3.2. An autosampler (Spark Triathalon) injected 20 μ l of the sample into the mobile phase. The pump (JASCO) then moved the mobile phase and sample past the column (BDS Hypersil C18, 150x3 mm, 3 μ particles) at a rate of 400 μ l/min. The column oven (JASCO) was kept at 30°C. Standards were in the range of 0.1-100 ng/ml and were run in duplicate before and after samples to ensure correct calibration. Data was stored and processed using Empower (Waters) from which it was exported into Microsoft Excel. An example of a chromatogram with the peaks representing DOPA, DOPAC, 5HIAA, dopamine, and HVA may be found in Figure 2.3. In accordance with Lilly's protocols, the additional peaks observed in the chromatograms were not identified once it had been established that they did not interfere with the primary analytes.

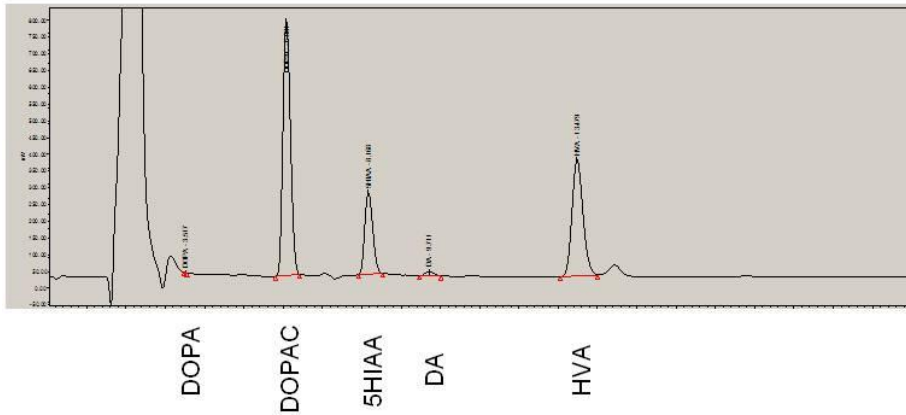
2.7. Operant tests

2.7.1. Testing in two lever boxes

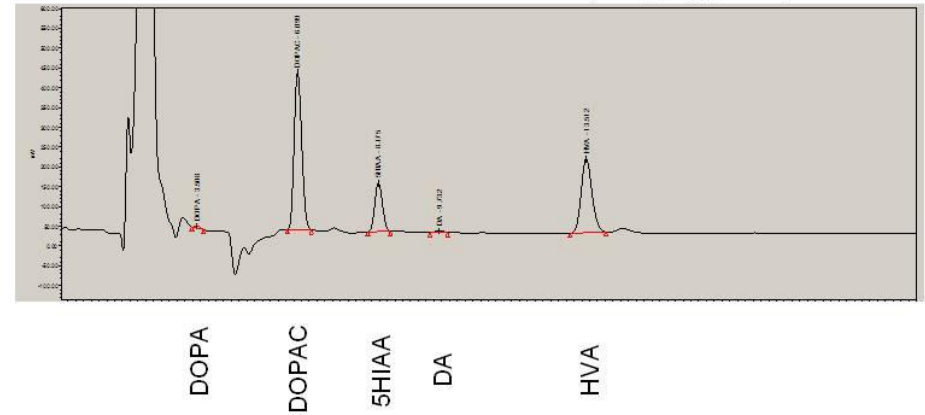
Apparatus

The two lever operant boxes were manufactured by Paul Fray Ltd (Cambridge, UK). The boxes were equipped with a house light in the ceiling, and two retractable levers. Pellets were delivered into a food hopper which was covered by a hinged Plexiglas panel positioned between the two levers. Behind the panel was a light which could be switched on to indicate

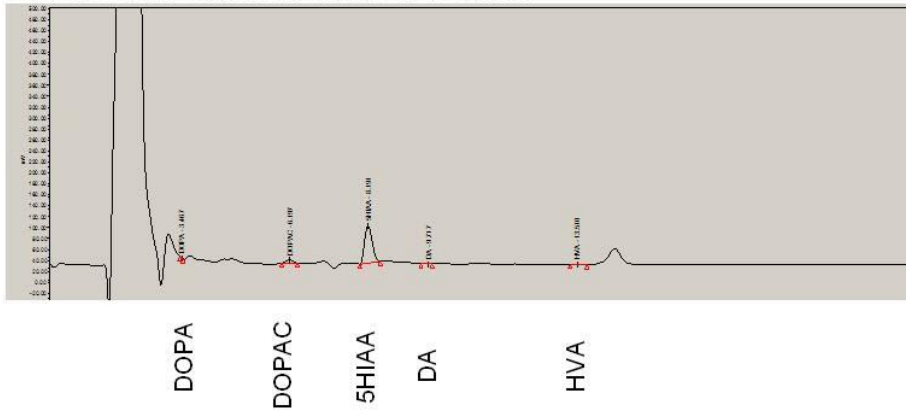
A. Intact striata before L-DOPA



B. Intact striata after acute L-DOPA (5mg/kg, s.c.)



C. Lesion striata before L-DOPA



D. Lesion striata after acute L-DOPA (5mg/kg, s.c.)

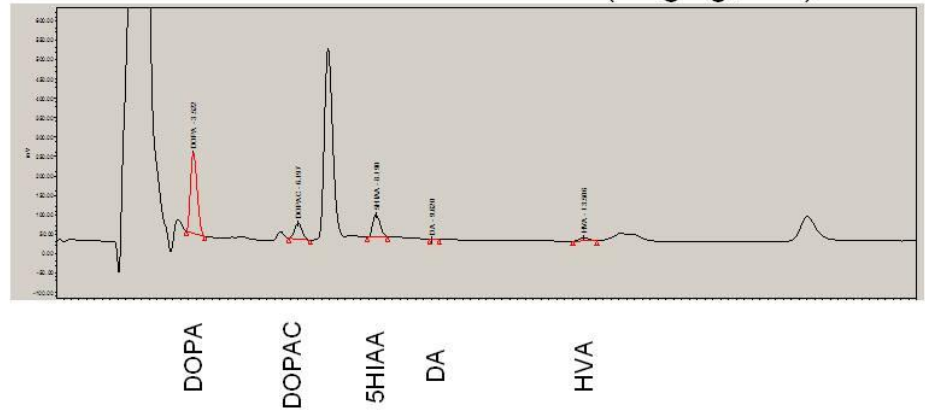


Figure 2.3. An example of a chromatogram from the microdialysis study described in Chapter 5. The graph shows the DOPA, DOPAC, 5HIAA, DA, and HVA peaks in the intact (A-B) and lesion (C-D) striata of a rat with a unilateral, 6-OHDA, MFB lesion before (A+C) and after (B+D) s.c. administration of 5 mg/kg L-DOPA. As explained in section 2.6.3, the additional peaks observed in the chromatograms were not characterised. The chromatograms were supplied by Jane Cooper at Eli Lilly & Co Ltd, Windlesham, Surrey. 5HIAA=5-Hydroxyindoleacetic acid, DA=dopamine, HVA=Homovanillic acid, s.c.=subcutaneous

the presence of a sucrose pellet. In addition, there were stimulus lights above each lever as well as the panel (Figure 2.4).

The boxes were 25 cm wide, 25 cm deep and 22 cm high. The panel measured 4.5 x 4.5 cm. The food hopper behind the panel was 4 cm deep and had a slight indentation in the

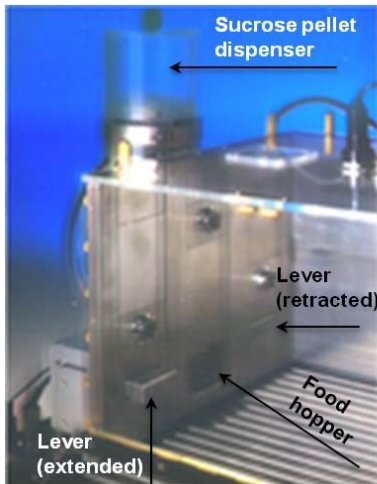


Figure 2.4. A photograph of a two lever operant box, with arrows indicating the location of the retractable levers, the food hopper from which sucrose pellets were delivered following a correct trial, and the sucrose pellet dispenser. A stimulus lights was located at the back of the Plexiglas panel covering the front of the food hopper (not visible in photo). The picture was provided by Cambridge Instruments (UK).

middle which caused the sugar pellets to naturally roll into the middle of the food hopper. The distance between the levers and the panel was 3 cm. The floor consisted of a metal grid which was located above a litter tray. The grids were placed 1 cm apart from each other.

2.7.2.1. Simple discrimination task

Task overview

Simple discrimination testing was conducted in two-lever operant chambers (Figure 2.4) with the house light turned on during testing. A normal session consisted of five blocks, each block consisting of ten trials each. On so called reversal days, rats were tested on six blocks, again consisting of ten trials each.

The rats initiated a trial by making a panel press, upon which both levers were presented. If a rat did not press either lever within 15 s, they received a “time-out” during which both levers retracted and the house and panel lights were turned off for 3 s. If the rat pressed the correct lever within the set time the panel light was switched on and a sucrose pellet (AIN-76A Rodent Tablet, TestDiet, UK) was delivered into the food hopper. If the

incorrect lever was pressed the rat received a time-out and no sucrose pellet was delivered. Following either lever press, both levers retracted as soon as the rat released pressure from the chosen lever. Both a correct response and a time-out was followed by a 2 s intertrial interval during which the house light was turned on.

The rats were rewarded for pressing one pre-determined lever for three days in a row after which they were tested on a so called reversal day. On the reversal day, rats were rewarded for pressing the same lever as previously in the first block, while the remaining five blocks rats rewarded rats for pressing the opposite (i.e. previously incorrect) lever. After the reversal day, rats were given three days of being rewarded for pressing the new “correct” lever. Accuracy was calculated as the number of correct trials over total trials and expressed as a percentage. Movement time was defined as the time elapsed between termination of a panel press and initiation of a lever press.

A normal session terminated after 25 min or 50 trials, whichever occurred first. On reversal days, i.e. when the contingencies changed, the session terminated after 30 min or 60 trials, whichever occurred first.

2.7.1.2.2. Pre-lesion training

Pre-lesion training was conducted in the same two lever boxes that were used for the simple discrimination task. Prior to commencing training, rats were food restricted for 1 week and exposed to sucrose pellets twice in their home cages to avoid neophobic reactions. During their first exposure to the operant boxes, the house and panel lights were both turned on and 30 sucrose pellets were automatically delivered into the food hopper after the rat had been placed there. Rats were allowed 30 min to consume the pellets and thereafter removed from the boxes.

On the second day of pre-lesion training, the house and panel lights were turned on and one single sucrose pellet was delivered into the hopper after the rat had been placed in the box. Whenever a rat entered the panel, another pellet was delivered thereby encouraging the rats to continue the panel pressing. Rats could obtain a maximum of 50 pellets during a 25 min session.

Once the rats panel pressed for 50 pellets in the 25 min session, lever press training commenced. During lever press training, the house light remained turned on but the panel light was only turned on following the delivery of a sucrose pellet. Pressing the panel caused one of the levers to be presented. The levers were presented in a pseudo-randomised order. Pressing the presented lever was always rewarded with the delivery of a sucrose pellet and illumination of the panel light. Once the rat collected the sucrose pellet, the panel light was

switched off. The session was terminated after either 50 lever presses or 25 min, whichever occurred first.

2.7.1.2.3. Sucrose consumption test

During the sucrose consumption test, which took place in the same two lever boxes as the simple discrimination task, one pellet was automatically delivered into the food hopper and the panel light was turned on after the rat was placed in the box. When the rat pressed the panel to enter the hopper and retrieve the pellet, the panel light was switched off and a 0 s or 5 s (depending on which version of the task that was being run) intertrial interval (ITI) commenced. Panel presses made during the 5 s ITI did not result in the delivery of further sucrose pellets but were recorded as perservative presses. At the end of the 5 s ITI, the panel light was turned back on and a panel press was again rewarded by delivery of another sucrose pellet. As before, delivery of a sucrose pellet was followed by a 0 s or 5 s ITI. The cycle continued for 25 min. The maximum number of pellets rats could obtain was set to 1000 but no rat reached this limit.

2.7.2. Testing in nine-hole operant boxes

Apparatus

The nine-hole operant boxes were manufactured by Paul Fray Ltd (Cambridge, UK). All boxes were fitted with a house light in the ceiling, and nine-holes into which the rats could poke. Sucrose pellets were delivered into a food hopper which was covered by a hinged Plexiglas panel and positioned on the wall opposite to the nine-holes. Behind the panel was a light which could be switched on to indicate the presence of a sucrose pellet (Figure 2.5). The holes that were not used were covered by plastic dummies.

The inside length of the boxes was 26 cm. The inside width of the boxes was 25 cm in the middle of the box, and 22.5 cm on either short side of the side of the box. The inside height of the boxes was 26 cm. The stimulus holes measured 2.3x2.3 cm, and were 3 cm deep. All stimulus holes were positioned 3 cm apart. The sensors detecting nose pokes into the stimulus holes were positioned 0.5 cm into the holes. The panel measured 8x8 cm, and the food hopper located behind the panel was 3.5 cm deep with a slight indentation in the middle that caused the sucrose pellets (AIN-76A Rodent Tablet, TestDiet, UK) to naturally roll into the middle of the food hopper. The floor consisted of a metal grid which was located above a litter tray. The grids were placed 1 cm apart from each other.

All hardware and software controlling the boxes was manufactured by Cambridge Cognition (Cambridge, UK).



Figure 2.5. A photograph of a nine-hole operant box, with arrows indicating the location of the stimulus holes in which rats responded during testing, the food hopper from which sucrose pellets were delivered following a correct trial, and the sucrose pellet dispenser. Stimulus lights were located in the end of each stimulus hole and at the back of the Plexiglas panel covering the front of the food hopper (not visible in photo). The picture is modified from Camden Instruments' website.

2.7.2.1. Lateralised Choice Reaction Time task

Task overview

During LCRT task testing, only three of the stimulus holes in the nine-hole boxes were exposed and the house light was by default turned on. Each trial began with the automatic illumination of the central stimulus light. The rats were required to maintain a nose poke into the illuminated hole for a variable length of time (200, 400, 600, or 800 ms) until either of the two lateral stimulus holes lit up for 50 ms. If the rat responded into the recently illuminated stimulus hole, the panel light was turned on and a sucrose pellet was delivered. If the rat terminated the nose poke before the end of the hold, responded into the incorrect hole, or neglected to make a response within 10 s, a “time-out” occurred during which the house light was turned off for 2 s. Following both time-outs and correct responses, a 2 s long intertrial period commenced after which another trial was automatically generated. Each session lasted for 30 min. There was no limit to the number of trials a rat could complete in one session.

A usable trial was defined as a trial where the rats maintained the hold into the central stimulus hole for the required length. Reaction time was defined as the time elapsed between the illumination of the lateral stimulus light and the discontinuation of the central nose poke.

Movement time was defined as the time elapsed between the discontinuation of a nose poke in the central hole and execution of a nose poke into a lateral stimulus hole. Only reaction and movement times following correct responses were reported.

2.7.2.2.4. Pre-lesion training

Prior to training, rats were food restricted to between 85% and 90% of their baseline weights. In instances where rats had not reached adulthood, their target weights were adjusted as previously described (2.1.3). To avoid neophobic reactions, rats were exposed to sucrose pellets twice in their home cages prior to pre-lesion training. Sucrose pellets were placed on the floor of their home cages in the evening and the cages were checked the following morning to confirm that the sucrose pellets had been consumed. During their first exposure to the operant boxes, rats were allowed one 15 min habituation session in the boxes. During this session, sucrose pellets were left in the three stimulus holes that were used in the LCRT task to encourage explorative behaviour, while the other holes were covered by plastic dummies.

During their second exposure to the boxes the rats completed a magazine training session, during which a sucrose pellet was automatically dropped into the illuminated food hopper in the beginning of the session and another pellet was dropped following each panel entry. During magazine training, the house light was kept on but all stimulus lights were off. Magazine training sessions were 15 min long.

After rats had learned to enter the magazine for sucrose pellets, central nose poke training commenced. During such training, the central stimulus hole was automatically illuminated in the beginning of each trial. Following a nose poke into the lit hole, the stimulus light was switched off, a sucrose pellet dropped into the panel and the panel light illuminated. A new trial was automatically generated when the rats' entered the food hopper. Central nose poke training sessions were 30 min long and followed by general nose poke training, where rats were trained to respond to any illuminated hole regardless of its location. General nose poke training sessions followed the same format as central nose poke training, but also included the use of the two lateral stimulus lights. At the beginning of a trial, one of the three stimulus lights was chosen by the software in a pseudo-randomised order, and automatically illuminated. Responding into the illuminated hole resulted in the delivery of a sucrose pellet as described previously.

Once the nose poke training was completed the rats were trained on the LCRT task. Initially, there was no minimum time the rats' had to maintain their response to the central stimulus light and the lateral lights were illuminated for 2 s. As the rats learned the task, the time which they had to maintain the central hold prior to onset of a lateral light cue was

gradually increased, and the length of time the lateral stimulus lights were switched on for was decreased. At the end of training, rats had to maintain their central nose pokes for 200, 400, 600, or 800 ms, and the lateral lights were only illuminated for 50 ms. The length of time rats had to maintain their central nose poke was determined by the software in a pseudo-randomised order, to ensure that lateralised nose pokes were made in response to the presentation of a visual cue and not merely executed following a pre-learned time interval.

2.8. Perfusion

Rats were deeply anaesthetised by i.p. administration of 1 ml Euthathal. Perfusion began once the rat had lost consciousness and no longer reacted to a tail pinch, toe pinch, or exhibited a blink reflex in response to gentle stimulation of the area around the eye. A cut was made across the rat's chest and rib case. Forceps were used to move the rib case thereby exposing the heart. Thereafter, the descending aorta was clamped, a needle attached to a motorised pump via a plastic tube was inserted into the apex of the heart, and a small incision was made to the top right atria to allow release of blood and PBS fluids. 1M PBS was then pumped through the circulatory system at a rate of 60 ml/min for 2 min. If there was still visible blood in the PBS leaving the system via the incision in the right atria following 2 min, PBS continued being pumped through the system until the fluids leaving the system were clear. The motorised pump was thereafter used to pump 1.5% paraformaldehyde (PFA) in 1 M PBS through the cardiovascular system for 4 min. If the limbs were not stiff by the end of the 4 min, further time was allowed until stiffness of the limbs was observed.

Once perfusion was complete, the brain was removed and placed in 1.5% PFA in 1M PBS overnight after which it was placed in 25% sucrose in 1M PBS and stored in a 4°C fridge.

2.9. Culling and collection of fresh striata

Rats were deeply anaesthetised by i.p. administration of 1 ml Euthathal. The procedure began once the animal had lost consciousness and no longer reacted to a tail pinch, toe pinch, or exhibited a blink reflex in response to light stimulation of the eye. Rats were decapitated using a rat guillotine, the brain removed and quickly placed on an ice-cooled petri dish to cool it. The brain was cut coronally at the level of the optical nerve using a razor blade and the hindbrain post-fixed in 4% PFA overnight after which it was transferred to sucrose. The cortex was peeled off the remaining brain, thereby exposing the striata. Fine point dissection forceps were used to cut around the striatum, which was then removed with the help of

curved dissection forceps. The ipsilateral and contralateral striata were placed in separate eppendorfs and immediately placed in dry ice. The tissue was then stored at -80°C until used.

2.10. Immunohistochemistry: Tyrosine Hydroxylase staining

The fixed brains were cut in 40 µm thick coronal sections using a freezing stage sledge microtome. Sections were stored in TBZ (0.1M TBS with 0.02% sodium azide) at 4°C until commencing immunohistological staining.

1 in every 6 sections was taken for histological staining. Following washes in 0.1M TBS the sections were incubated in quench (3% hydrogen peroxide, 10% methanol, in distilled H₂O) for 5 min before further TBS washes were conducted. Non-specific binding was blocked for 1 h using TBS with 0.1% Triton x-100 (Sigma-Aldrich, UK) and 3% horse (cat no 1650) or goat (cat no 16210) serum (both Invitrogen, UK). Sections were then incubated in TBS with 3% serum, 0.1% triton X-100, and 1:2000 primary anti-TH antibody (MAB318 or MAB318, Millipore, UK). Sections were left to incubate on a shaker, overnight in room temperature. The following day, sections were washed in TBS before incubated for 2 h in TBS with serum and 1:200 secondary antibody (BA9010 or BA1000, Vector, UK). The incubation was conducted on a shaker in room temperature. Sections were then washed in TBS before incubation in ABC kit (DAKO, UK) solution (0.5% solution A, 0.5% solution B, 1% goat serum in 1M TBS). Following a 2h incubation, sections were given further TBS washes, followed by 2x5min washes in 0.05M TNS, pH 7.4. The section bound antibody was visualised using 3-3'-diaminobenzadine (DAB, Vector Laboratories). During visualisation, the sections were incubated in TNS and 0.5% DAB with 3% of 30% hydrogen peroxide (VWR) added to initiate the reaction. The reaction was stopped by washing the sections in TNS. The stained sections were hand-mounted on gelatinised microscope slides, allowed to air-dry overnight, dehydrated in ascending concentrations of alcohol (75-100%, Fisher Scientific), cleared in xylene (VWR), and finally cover-slipped using DPX mountant.

An illustrative example of a nigra stained for tyrosine hydroxylase using the protocol described above is provided in Figure 2.6.

2.11. Cell counting

Anatomical structures were defined according to Paxinos & Watson (1986). Distinction between the SNc and VTA was made by defining the VTA as the TH+ cell body containing area medial to the MT tract and lateral to the fasciculus retroflexus. Cell bodies staining

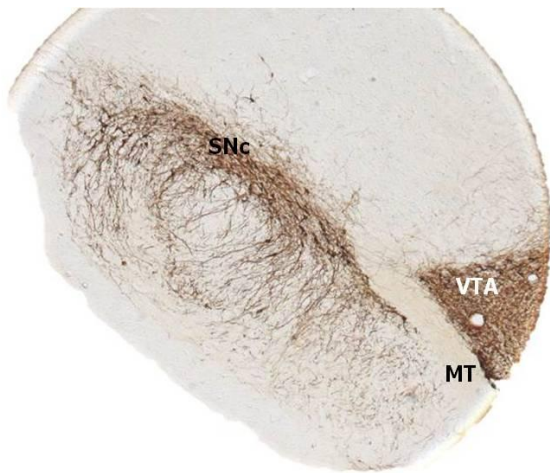


Figure 2.6. Example of an intact nigra stained for tyrosine hydroxylase using the protocol described in section 2.10. MT=Medial terminal nucleus, SNc=Substantia nigra pars compacta, VTA=Ventral tegmental area.

positive for TH in the SNc and VTA were manually counted using a microscope with a 10 x magnification at the level where the mammillotegmental tract separated the two regions. One section was counted per rat, All TH+ cell bodies in the SNc and VTA of the section were counted, and the cell bodies in the ipsilateral side were expressed as a percentage of the intact side. It is recognised that the method does not provide an absolute number of cell bodies such as stereotactic counting would. However, in the experiments reported in this thesis cell counting was conducted to confirm presence of a lesion and expressing dopamine loss as a percentage of intact side was therefore sufficient. Rats with <90% dopamine loss in the ipsilateral SNc following a MFB lesion were considered not to have full lesions. These were therefore excluded from further analysis with the exception of (i) Experiment 2 where data from rats with <90% dopamine loss in the SNc was included as the aim of the experiment was to compare motor performance of rats with <90% and $\geq 90\%$ SNc dopamine loss and (ii) Experiment 3 which used intra-striatal lesions that are known to induce less pronounced loss of dopaminergic cell bodies (Yuan *et al*, 2005) and where no cut-off was used.

2.12. Optical density

Optical density was used to measure striatal dopamine denervation in Experiments 3, 4, 5 and 9. Optical density was measured from one section per rat. Pictures showing both the ipsilateral and contralateral striata were taken using a Leica DFC420 camera and Leica application v3.6 software. Photos were converted into black-and-white 8-bit format and optical density in the striatum measured using Image J v1.42 software (National Institute of

Health, U.S.A.). A grey scale was used to convert grey scale measurements into arbitrary optical density measures. The optical density of the corpus callosum was subtracted from the optical density values of the ipsilateral and contralateral striatum. The normalised optical density measures were then used for measuring the percentage loss of striatal optical density and for subsequent statistical analyses.

2.13. Western blot

All Western blot analysis reported in this thesis was conducted in Dr. Fabrizio Gardoni's (Department of Pharmacology, Milan University, Italy) lab who also quantified the Western blot data and provided the author of this thesis with numerical data for statistical analysis.

Subcellular fractionation of striatal tissue was performed as described in Gardoni *et al* (2006) with minor modifications. Striata were homogenized in ice-cold sucrose 0.32 M containing 1 mM HEPES, 1 mM MgCl₂, 1 mM EDTA, 1 mM NaHCO₃, 0.1 mM PMSF, at pH 7.4. The homogenized tissue was centrifuged at 1000 g for 5 min. The resulting supernatant was centrifuged at 13000 g for 15 min to obtain a crude membrane fraction. The pellet was resuspended in buffer containing 75 mM KCl and 1% Triton X-100 and centrifuged at 100 000 g for 1 h. The final pellet, referred to as TIF, was homogenized in a glass-glass potter in 20 mM HEPES and stored at -80°C until processing. TIF was used instead of the classical Post-Synaptic Density, because the amount of the starting material was very limited. All purifications were performed in presence of a complete set of protease (Complete) and phosphatase (PhosSTOP) inhibitors (Roche Diagnostics, Basel, Switzerland). Protein content of the samples was quantified using the Bio-Rad (Hercules, CA, USA) protein assay. After measuring protein concentration, all samples were standardized at 1 µg/ul concentration and 20 µg/sample loaded in each lane. Western Blot analysis was performed on the TIF using monoclonal antibodies raised against N-Methyl-D-aspartic acid (NMDA) glutamate receptor subunits NR2A (Zymed, San Francisco, USA) and NR2B (NeuroMab, Davis, USA). Quantification of Western blotting analysis was performed by means of computer-assisted imaging (Quantity-One® System; Bio-Rad) after normalization on tubulin levels.

2.14. Statistical analysis

All statistical analysis was conducted using SPSS v21.0. In instances of missing values, these were replaced using the series mean. Details on statistical tests are specified in the method section of each experiment.

3. Strain Differences & Lesion Screening

Background: The chapter describes two experiments that informed methodological decision making in later thesis experiments.

Aim: Two experiments were conducted to determine (i) whether Lister Hooded rats could be used in experiments studying the behavioural effects of LID onset, (ii) whether lesion rats that had been chronically treated with L-DOPA could be tested in operant chambers following acute L-DOPA administration without the presence of disabling AIMs, and (iii) whether non-pharmacological behavioural tests could be used to predict lesion success in the unilateral 6-OHDA MFB rat.

Methods: Experiment 1 compared Lister Hooded and Sprague Dawley rats in terms of their AIMs following chronic L-DOPA treatment, their motor response to acute L-DOPA, and their performance on a simple discrimination task following acute administration of L-DOPA or saline. Experiment 2 used behavioural and histological data from a pilot experiment to determine the relationship between dopamine loss in the SNc and VTA and performance on non-pharmacological motor tests. These findings were compared with behavioural and histological data collected in later thesis experiments.

Results: Data from Experiment 1 suggested that (i) Lister Hooded rats can be used in L-DOPA experiments instead of the more commonly used Sprague Dawley strain, and (ii) lesion rats that have received chronic L-DOPA treatment can be tested in operant chambers following acute administration of a low (1 mg/kg) L-DOPA dose without disabling dyskinesias interfering with operant performance. Data from Experiment 2 suggested that the non-pharmacological cylinder test is preferable to amphetamine induced rotations when screening lesion success in psychopharmacological experiments.

3.1. Introduction

3.1.1. Pigmented versus albino rat strains

A primary aim of this thesis project was to test the effect of acute and chronic L-DOPA on non-motor function in the 6-OHDA rat model of PD. This was planned to be accomplished, in part, by using the operant LCRT task (section 2.7.2.2). The dyskinetic and motor effects of L-DOPA are typically studied using albino rat strains, such as the Sprague Dawley rat (e.g. Monville *et al*, 2005, Breger *et al*, 2013). However, pigmented strains such as the Lister Hooded rat have better vision (Prusky *et al*, 2002) and are therefore preferred in operant paradigms reliant on the presentation of visual cues such as the LCRT task (e.g. Carli *et al*, 1985).

There are known differences between pigmented and albino rats' spontaneous activity levels and their performance on behavioural tests such as object discrimination, spatial learning, and autoshaping (Andrews *et al*, 1995; Nakagawara *et al*, 1997). Rat strains also differ in a range of parameters that could affect the response to dopaminergic treatment such as basal levels of dopamine, dopamine transporter (DAT), and the expression of striatal D1 and NMDA receptors (Sziraki *et al*, 2001; Zamudio *et al*, 2005; Lei *et al*, 2009) which are implicated in LID (Mela *et al*, 2012; Gardoni *et al*, 2012; Sawada *et al*, 2010). Whilst it is unknown whether there are strain differences in cortico-striatal plasticity, which is also implicated in LID (Picconi *et al*, 2003), Lister Hooded rats are known to differ from the albino Wistar strain in e.g. hippocampal plasticity (Manahan-Vaughan & Schwegler, 2011).

Because of the behavioural and physiological differences between albino and pigmented rat strains, the possibility that they may also differ in their motor and dyskinetic response to L-DOPA could not be excluded. The LCRT task, where rats respond to visual stimuli (Carli *et al*, 1985) and where a high level of visual acuity is required, was to be utilised in later experiments. The pigmented Lister Hooded strain has previously been used in in-house LCRT task experiments (e.g. Dowd & Dunnett, 2004), and would because of their higher visual acuity (Prusky *et al*, 2002) be preferable to albino strains in LCRT task studies. However, Lister Hooded rats are not typically used in L-DOPA studies. Previous in-house experiments on L-DOPA and LID have instead utilised Sprague Dawley rats (e.g. Monville *et al*, 2005, Breger *et al*, 2013). Therefore, before proceeding with psychopharmacological experiments where L-DOPA was administered to Lister Hooded rats before LCRT task

testing, it was desirable to test whether there were gross strain differences in Sprague Dawley and Lister Hooded rats' dyskinetic, motor, and operant response to L-DOPA.

3.1.2. Pharmacological versus non-pharmacological lesion screening tests

Another issue which warranted further study was the way in which lesion extent would be assessed following recovery from unilateral 6-OHDA lesions. A successful unilateral lesion causes marked asymmetry in the dopamine levels of the intact and lesion striata, which is reflected in an ipsilateral bias of rats' movement (Ungerstedt, 1968). The bias may be enhanced pharmacologically, using apomorphine or amphetamine (e.g. Matsuda *et al*, 1995; Ungerstedt & Arbuthnott, 1970). The dopamine agonist apomorphine induces a contralateral rotational bias, as 6-OHDA lesions are associated with sensitisation of dopamine receptors in the lesion striatum. Amphetamine, on the other hand, causes dopamine release from dopaminergic terminals whilst simultaneously blocking dopamine reuptake. This increases the already existent dopamine asymmetry between the lesion and intact striata of rats with unilateral 6-OHDA lesions and enhances their ipsilateral rotational bias (Ungerstedt & Arbuthnott, 1970; Costall *et al*, 1976; Carboni *et al*, 1989). The ability to pharmacologically enhance unilaterally lesion rats' rotational bias has led to apomorphine and amphetamine-induced rotations being commonly used to assess lesion extent in research utilizing the rat 6-OHDA model of PD (Smith *et al*, 2010).

However, there are limitations to using apomorphine- and amphetamine-induced rotations as lesion screening tests. Apomorphine exposure induces a long-lasting sensitisation of apomorphine-induced rotations (Klug & Norman, 1993) and has been shown to increase the magnitude of later AIMs (Delfino *et al*, 2004). As it alters both later motor behaviour and AIMs, it was not considered appropriate for use in experiments exploring the effects of LID onset on motor and non-motor function. Furthermore, while apomorphine causes a robust rotational response in rats with >90% striatal dopamine loss, it does not detect less pronounced lesions (Hefti *et al*, 1980) and is therefore not suitable for e.g. the intra-striatal lesion model utilised in Chapter 4. Acute amphetamine does cause a turning bias in rats with <90% striatal dopamine loss (Hefti *et al*, 1980). Unlike apomorphine, the net rotational bias observed following acute amphetamine also correlates significantly with striatal dopamine loss (Hudson *et al*, 1993). However, recent data has queried whether the long-term effects of the drug make it an inappropriate lesion screening method for cognitive and psychopharmacological experiments.

For example, when Nelson & Killcross (2006) investigated the effect of amphetamine on habit formation, rats that were exposed to d-amphetamine (2 mg/kg for seven days) were found to conduct more magazine entries and lever presses when tested on a random-interval schedule post-satiation than saline treated controls did. As such, the data suggested that amphetamine exposure made rats prone to revert to habitual responses, rather than exhibiting goal-directed behaviours (Nelson & Killcross, 2006). This hypothesis was supported by a study conducted by a separate group in which rats that had been exposed to d-amphetamine (2.5 mg/kg for five days) prior to training on a random interval schedule failed to show a decrease in lever presses following devaluation, again suggestive of an amphetamine-driven switch from goal directed to habitual behaviour (Nordquist *et al*, 2007). In addition, Nordquist and colleagues (2007) observed that, prior to satiation, amphetamine-treated rats made more presses on the reinforced lever than saline controls did on a random ratio task without differing in their lever presses on a non-reinforced lever. It was suggested that this phenomenon represented an increase in the motivational value of the sucrose reward following amphetamine priming (Nordquist *et al*, 2007). Together, the studies by Nelson & Killcross (2006) and Nordquist and colleagues (2007) suggest a replicable effect of repeated amphetamine exposure on habit formation.

There is also reason to believe that even a single amphetamine exposure may have long-term effects on the dopaminergic system. Rats that have been exposed to one injection of 5 mg/kg amphetamine show an increased locomotive response to later injection of 1 mg/kg amphetamine (Vanderschuren *et al*, 1999). Relative to saline controls, these rats also show increased locomotor response to acute cocaine, and a trend ($p=0.06$) towards an increase in their locomotor response to a D2 agonist (Vanderschuren *et al*, 1999). Furthermore, *in vitro* data show that a single exposure to amphetamine (i) increases the levels of electrically evoked dopamine and acetylcholine release *in vitro* from tissue slices obtained from the striatum, NAcc, and prefrontal cortex (Vanderschuren *et al*, 1999), (ii) increases the striatal 5-HIAA/5-HT ratio (Cadet *et al*, 2010), and (iii) alters the expression of a range of genes in the intact as well as the dopamine denervated striata, including an increase in c-Fos (Cadet *et al*, 2010).

Together, the abovementioned studies imply that repeated administration of amphetamine as part of lesion screening could affect subsequent non-motor behaviour and potentially confound data obtained from operant tasks, particularly in paradigms based upon stimulus-response associations. Furthermore, even a single exposure to amphetamine could affect later neurochemical or behavioural responses to dopaminergic drugs. For these reasons,

amphetamine was considered inappropriate for screening lesions in experiments testing the effect of L-DOPA on subsequent motor and non-motor behaviour. Therefore, Experiment 2 analysed histological and behavioural data gathered from a pilot experiment in order to identify non-pharmacological screening tests that could be used in later thesis experiments.

3.2. Experiment 1: Assessing Strain Differences in L-DOPA Response

3.2.1. Introduction

The primary purpose of the experiment was to determine whether Lister Hooded rats could be used in place of Sprague Dawley rats in later experiments including both LID induction and operant testing. However, the opportunity was seized to simultaneously test whether (i) it was possible to administer acute L-DOPA to lesion rats that had previously been chronically treated with L-DOPA prior to testing in operant boxes without AIMs interfering with operant behavioural output, (ii) if acute L-DOPA impacted on lesion rats' performance on a simple discrimination and reversal learning task.

LID is associated with a range of changes to the dopaminergic system, including sensitization of D1 receptors (Aubert *et al*, 2005) which are implicated in the expression of AIMs (e.g. Mela *et al*, 2012). Following LID onset, animals will exhibit AIMs following subsequent acute L-DOPA administration. If such AIMs were to occur during testing in operant chambers they could interfere with purposeful motor actions, confound the data, and increase the risk of injuries. It was therefore necessary to determine if a low dose of acute L-DOPA could induce sufficient motor activation so as to be functional in rats with previous history of chronic L-DOPA treatment, without inducing severe AIMs.

When testing whether it was possible to conduct operant testing following acute administration of a low L-DOPA dose, a simple discrimination and reversal learning task which was hypothesized to rely on the dopaminergic system was utilised. The task took place in two-lever boxes and rewarded rats for pressing one, over the other, lever. Following three days of testing, the contingencies were changed so as to reward rats for pressing the opposite lever. Simple discrimination and reversal learning both depend on striatal function, but recruit different part of the striatal system. Lesions to the dorsolateral striatum, which is connected to prefrontal motor cortex, disrupt acquisition of a simple discrimination task (Featherstone *et al*, 2004; Robbins *et al*, 1990). Conversely, reversal learning is believed to be reliant on the ventral striatum and ventral prefrontal cortex (Clarke *et al*, 2004). Based on previously published data suggesting a disruptive effect of dopamine depletion on performance on a

discrimination task (e.g. Robbins *et al*, 1990) it was hypothesised that lesion rats would be impaired relative to intact controls when acquiring the simple discrimination task utilised in this experiment. Moreover, because the lesions were unilateral it was further hypothesized that the deficit would only manifest itself when lesion rats were reinforced for responding on the lever contralateral to the lesion.

Following dopamine loss, it has been suggested that performance on simple discrimination and reversal learning tasks is affected by acute L-DOPA (Cools, 2006). Acute L-DOPA administration has been shown to reinstate the ability to acquire a simple discrimination task in dopamine deficient mice which were otherwise unable to learn the task (Robinson *et al*, 2007). Conversely, acute L-DOPA also impairs accuracy on reversal learning tasks (Clarke *et al*, 2004; Cools, 2001). The disparity in the effect of acute L-DOPA on simple discrimination and reversal learning has been suggested to relate to the drug's ability to mimic the neural "reward signal", a signal consisting of phasic dopamine release that typically occurs following delivery of a US (Schultz, 2010). By mimicking the reward signal, which is lost or attenuated following dopamine denervation, acute L-DOPA has been suggested to enhance learning dependent on positive reinforcement in PD patients (Cools, 2006). However, the elevated striatal dopamine levels that follow acute L-DOPA administration have been suggested to simultaneously mask the depression in dopamine activity that typically occur following omission of an expected reward (Frank *et al*, 2004; Schultz, 2010; Cools, 2006). Based on current literature, it was therefore hypothesised that acute L-DOPA administration would restore the dopamine reward signal that was expected to be lost or reduced when lesion rats performed correct contralateral responses, while simultaneously masking the depression in dopamine activity that should occur when an expected reward following a correct response was omitted (i.e. when switching contingencies). Hence, during operant testing it was hypothesized that acute L-DOPA would (i) improve lesion rats' performance when lesion rats were rewarded for making contralateral responses, and (ii) impair lesion rats' reversal learning.

As previously stated, excitotoxic lesions to the ventral striatum have been shown to impair reversal learning (Clarke *et al*, 2004). In addition, dopamine loss in the NAcc (which forms part of the ventral striatum) also reduces responding for food pellets in progressive ratio tasks, which are commonly used to measure rats' motivation to work for food or drug rewards (e.g. Hamill *et al*, 1999). MFB lesions decrease dopamine levels in both the dorsal and ventral striatum (Deumens *et al*, 2002). It was therefore possible that the MFB 6-OHDA lesions used in this experiment would decrease rats' motivation to work for sucrose pellets.

Acute L-DOPA could further affect motivation, as the L-DOPA doses required to offer therapeutic relief of motor symptoms (mediated by the dorsal striatum) are believed to simultaneously over stimulate the less denervated ventral striatum (Cools, 2006; Hamill *et al*, 1999). Therefore, at the end of the simple discrimination task, the current experiment also tested whether there was an effect of 6-OHDA MFB lesions, and acute L-DOPA on the number of sucrose pellets consumed by rats in a sucrose pellet test.

3.2.1.1. Aims

The aim of the experiment was to (i) test for strain differences in Sprague Dawley and Lister Hooded rats' motor and dyskinetic response to acute L-DOPA, (ii) confirm whether lesion rats that had been chronically treated with L-DOPA treatment could be tested in operant chambers following a low dose of acute L-DOPA without the interference of AIMs, and (iii) test whether a low dose of acute L-DOPA affected learning on a simple discrimination and reversal learning task or (iv) the number of freely available sucrose pellets consumed by lesion rats.

3.2.2. Methods

3.2.2.1. Experimental design

A subgroup of Lister Hooded and Sprague Dawley rats was given unilateral 6-OHDA lesions to the MFB and then chronically treated with L-DOPA until stable AIMs developed. Following a three day wash-out period, the effect of acute L-DOPA on the vibrissae and adjusting step tests, as well as on rats' spontaneous activity levels, was measured. Rats were then tested on a simple discrimination task, and a pellet consumption test following acute administration of saline or 1 mg/kg L-DOPA (Figure 3.1).

3.2.2.2. Procedure

Female Lister Hooded (n=21) and Sprague Dawley (n=20) rats were food restricted for 1 week before undergoing magazine and lever press training in two-lever operant boxes (2.7.2.2.4). All rats completed training within 14 days.

Subgroups of the Lister Hooded (n=13) and Sprague Dawley (n=12) rats were given unilateral 6-OHDA MFB lesions using 6-OHDA (section 2.2.2), leaving the remaining Lister Hooded (n=8) and Sprague Dawley (n=8) rats as intact controls. Following recovery, lesion extent was assessed using amphetamine-induced rotations (section 2.4.6.2). All lesion rats were then administered 10 mg/kg L-DOPA daily for two weeks during which period they were scored for AIMs (section 2.5). At the end of the chronic L-DOPA treatment an

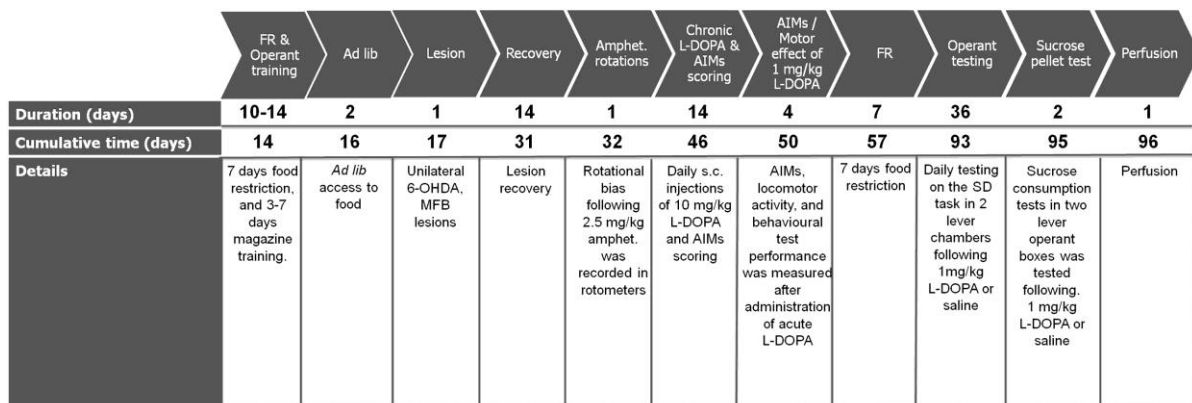


Figure 3.1. A graphical representation of the experimental design of Experiment 1. The figure specifies both the duration of the individual testing phases, and the overall number of days in the experiment. Ad lib=Ad libitum, AIMs=Abnormal involuntary movements, Amphet.=Amphetamine, FR=Food restriction, MFB=Medial forebrain bundle, s.c.=subcutaneous, SD=Simple discrimination

additional AIMs scoring session was conducted. In this session, AIMs were recorded following administration of 1 mg/kg L-DOPA. At the same time, the effect of 1 mg/kg L-DOPA on activity levels was measured using activity cages (section 2.4.2). Rats were allowed a 30 min habituation period in the activity boxes before s.c. L-DOPA administration, after which the number of beam breaks made was automatically recorded by the system for 60 min. Note that the purpose of the test was to determine whether or not the chosen L-DOPA dose would induce a behavioural effect in lesion rats. For this reason, and due to a restricted number of available activity cages, only lesion rats were tested. Furthermore, to better understand the effect of 1 mg/kg L-DOPA on motor function, lesion rats' performance on the adjusting step and vibrissae tests (2.4.3 and 2.4.8) was measured following administration of L-DOPA ("on" condition) or saline ("off" condition).

Intact and lesion rats were then food restricted for one week and tested on a simple discrimination task in two-lever operant boxes (section 2.7.1) where their ability to discriminate between a reinforced and non-reinforced lever was measured. Approximately half of the lesion Lister Hooded (n=7) and Sprague Dawley rats (n=5) were injected s.c. with 1 mg/kg L-DOPA 20 min prior to testing. The remaining lesion rats and all intact rats were given a s.c. saline injection of 1 ml/kg. At the end of the operant testing rats were tested in two versions of a pellet consumption test, in a counter balanced order. The pellet test took place in the same two-lever operant boxes as before and is further described in section

2.7.1.2.3. Briefly, pressing the illuminated panel resulted in the delivery of a sucrose pellet and the extinction of the panel light. Following an inter-trial interval (ITI), the panel was re-illuminated and the rats could panel press to obtain another sucrose pellet. Presses made during the ITI did not produce a sucrose pellet. The two versions differed in the length of the ITI, which was either 0 s or 5 s. Previous observation of lesion rats had suggested that they could consume one sucrose pellet in less than 5 s. Therefore, a 5 s ITI was hypothesized to be less sensitive to an effect of 6-OHDA lesions on motor function than a 0 s ITI. Specifically, in the 0 s ITI version, it was expected that if both groups continuously panel pressed for sucrose pellets as fast as they could, the lesion induced motor impairment would still cause the lesion cohort to obtain overall fewer sucrose pellets than the intact group. Therefore, if lesion rats were to perform worse than intact rats on the 0 s ITI but not the 5 s ITI version of the task it could be hypothesized that the difference between lesion and intact rats was driven by a motor deficit.

At the end of testing, rats were perfused (section 2.8) and their brains used for TH immunohistochemistry (section 2.10). Lesion extent was determined by counting the surviving TH+ cell bodies in the SNc and VTA (section 2.11).

3.2.2.3. Statistical Analysis

Neither SNc nor VTA dopamine denervation data showed a normal distribution. Whereas the VTA denervation data showed normal distribution after the data values were converted into the square root of the original values, it was not possible to transform the SNc dopamine denervation data to meet normal distribution. Therefore, the transformed VTA dopamine denervation data were analysed using a one-way ANOVA whereas the SNs dopamine denervation data were analysed using a non-parametric Kruskal Wallis test. Both tests compared the four experimental groups' (Sprague Dawley or Lister Hooded rats administered acute L-DOPA or saline during motor and operant tests) degree of dopamine denervation expressed as a percentage of the intact side. Striatal optical density was analysed in two separate non-parametric tests. First, the raw optical density measures from the ipsilateral versus contralateral striatum were compared using a Wilcoxon test to confirm that the lesion had reduced TH+ staining in the lesion striata. Second, a Kruskal-Wallis test was used to test whether the experimental groups differed in the percentage loss of ipsilateral striatal TH+ immunoreactivity induced by the lesion.

AIMs scores were analysed using a repeated measures ANOVA with Strain as between subject factor and Dose as a within subject factor.

Baseline activity data were calculated as the average activity recorded during the habituation period and analysed using a one-way ANOVA. The effect of L-DOPA on spontaneous locomotion was analysed including all time points and using a repeated measures ANOVA with Strain as between subject factor, and Time Point as a within subjects factor.

Data from the motor behaviour tests were analysed using a repeated measures ANOVA with Strain as a between subject factor, and Drug and Side as within subjects factors. As previously described, this data set only included data from lesion rats (n=25).

Data from the simple discrimination task were analysed using a repeated measures ANOVA with Strain, Lesion, and Drug as between subject factors, and Side, Day, and Block as within subject factors. Block referred to a set of 10 consecutive trials, and was used as an indicator of within-session learning. Each trial consisted of 5 blocks, i.e. 50 trials.

Pellet consumption data were analysed using a repeated measures ANOVA with Strain, Lesion, and Drug as between-subject variables, and the length of the ITI as a within-subject variable.

3.2.3. Results

3.2.3.1. Lesion extent

When counting the number of dopaminergic cell bodies, (described in section 2.11), a median of 118 (1st and 3rd quartile = 107 and 135) and 120 (1st and 3rd quartile = 103 and 152) TH positive cell bodies were counted in the intact SNc and VTA respectively (numbers from one brain section). These numbers are in line with what has been observed in other in-house studies using the same immunohistological protocol, and the same histological and microscope equipment (unpublished data).

The lesion induced a profound loss of TH positive cells bodies in both the ipsilateral SNc (Figure 3.2A) and the ipsilateral VTA (Figure 3.2B). A further analysis comparing the percentage loss in the ipsilateral relative to the contralateral hemisphere demonstrated that the four groups (Lister Hooded/Sprague Dawley tested on/off acute L-DOPA during motor

and operant testing) did not differ significantly in their denervation of the ipsilateral SNc (H=4.72, df=3, n.s.) or VTA ($F_{3,24}=1.57$, n.s.).

In addition to SNc and VTA denervation, the lesion also produced a significant decrease in the optical density measured in the lesion relative to the intact striatum (Figure 3.2C; $Z=-4.37$, $p<0.001$). A further analysis based on the percentage decrease in ipsilateral optical density showed that the striatal denervation did not differ significantly between the groups (H=7.71, df=3, n.s.).

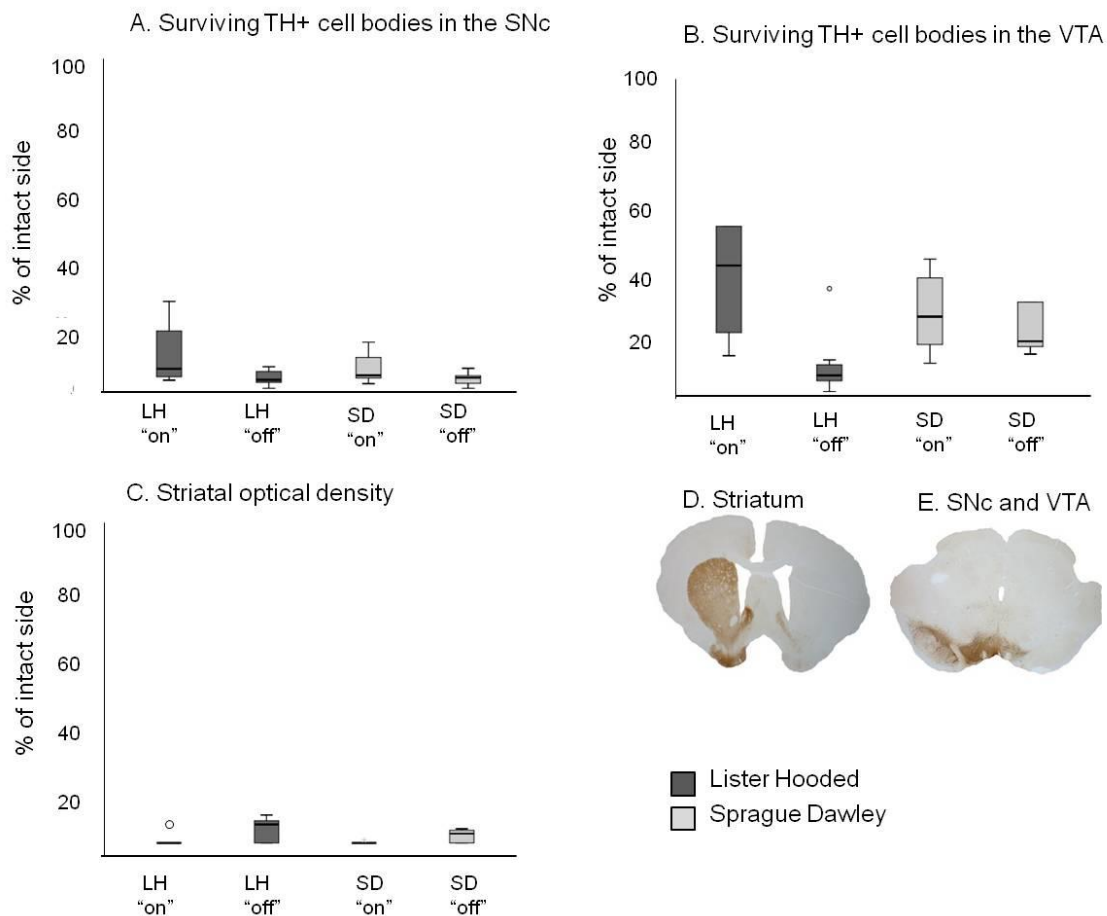


Figure 3.2. The median surviving TH+ cell bodies in the ipsilateral SNc (A) and VTA (B), and the mean striatal optical density (C), all shown as a percentage of the intact side, of the lesion Lister Hooded (n=13) and lesion Sprague Dawley (n=12) cohorts. The photos show the striatum (D), the SNc, and the VTA (E) of a lesion rat included in the experiment. "On" and "off" refers to groups administered 1 mg/kg acute L-DOPA ("on") or 1 mg/kg saline ("off") prior operant testing. The box plots show the median and interquartile ranges, and circles represent outliers. LH=Lister Hooded, SD=Sprague Dawley, SNc=Substantia nigra pars compacta, TH=Tyrosine hydroxylase, VTA=Ventral tegmental area.

3.2.3.2. Dyskinetic response to L-DOPA

Following chronic L-DOPA, all lesion rats developed LID. As expected, AIMs scores were significantly lower following administration of 1 mg/kg, than 10 mg/kg, L-DOPA (Figure 3.3; Dose: $F_{1,23}=9.26$, $p<0.001$). Lister Hooded rats showed significantly higher AIMs than Sprague Dawley rats, especially following administration of 10 mg/kg L-DOPA (Strain: $F_{1,23}=7.89$, $p<0.05$; Dose x Strain: $F_{1,23}=6.28$, $p<0.05$; Simple Effect: $F_{1,23}=7.56$, $p<0.05$).

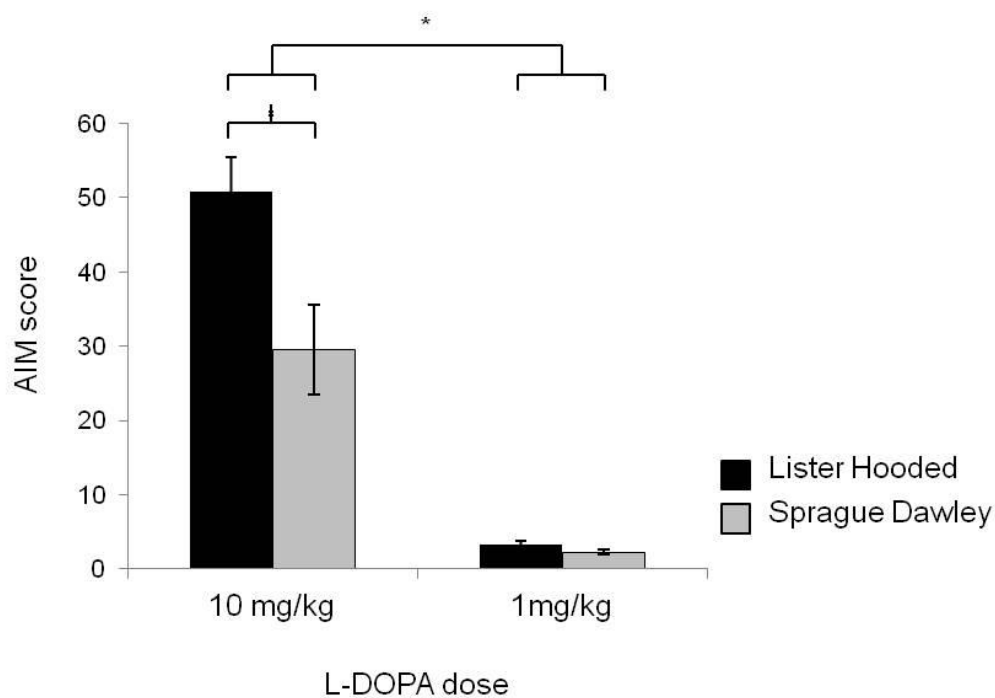


Figure 3.3. The average cumulative AIMs scores of the lesion Lister Hooded (n=13) and lesion Sprague Dawley (n=12) group following administration of 10 mg/kg and 1 mg/kg L-DOPA. The error bars show the standard error of the mean. AIMs=Abnormal involuntary movements.*Effect of Dose; †Effect of Strain, † $p<0.05$, * $p<0.05$

3.2.3.3. Effect of acute L-DOPA on motor function

The two strains' locomotor response to 1 mg/kg acute L-DOPA was measured both by comparing their average baseline activity levels (one-way ANOVA) and by analysing the number of beam breaks made at all testing time points before and after L-DOPA injection (repeated measures ANOVA). There was no significant difference between the baseline number of beam breaks made by the Lister Hooded and Sprague Dawley strains (Figure 3.4A; $F_{1,23}=0.30$, n.s.). Neither was there a significant difference in locomotor activity when comparing all the beam breaks made during the session (Strain: $F_{1,23}=0.30$, n.s.). Both strains

showed a significant change in the number of beam breaks made at different times of testing (Time point: $F_{21,483}=9.74$, $p<0.001$; Time point x Strain: $F_{21,483}=0.67$, n.s.). However, visual inspection of the data did not reveal a clear increase in activity levels post L-DOPA injection. Instead the fluctuations related to the increased locomotion displayed by rats when first placed in the chambers, which likely reflected explorative behaviour of a novel environment.

Rats' forelimb motor function was tested using the adjusting step test. Lesion Sprague Dawley and Lister Hooded rats demonstrated a similar contralateral impairment on the adjusting step test (Figure 3.4B; Side: $F_{1,23}=31.08$, $p<0.001$; Strain: $F_{1,23}=1.62$, n.s.; Side x Strain: $F_{1,23}=2.19$, n.s.).

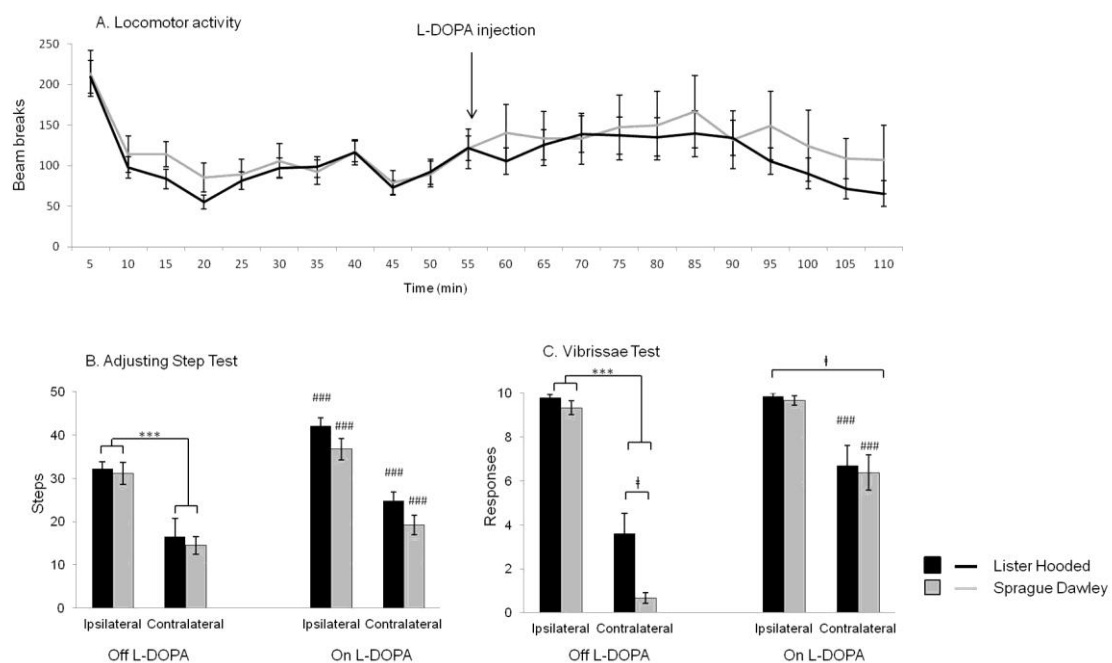


Figure 3.4. Mean locomotor activity before and after acute L-DOPA administration (A), together with the mean performance on the adjusting step (B), and vibrissae (C) tests of lesion Lister Hooded (n=13) and Sprague Dawley (n=12) rats that had previously received chronic L-DOPA treatment and were tested following acute administration of 1 mg/kg L-DOPA or saline. The error bars show the standard error of the mean. *Significant difference between ipsilateral and contralateral performance in rats tested off L-DOPA, #Significant difference between responses made when tested on versus off L-DOPA, †Significant difference between Lister Hooded and Sprague Dawley rats' performance. † $p<0.05$, * $p<0.001$, ### $p<0.001$. † $p<0.05$, *** $p<0.001$, ### $p<0.001$.**

Acute administration of 1 mg/kg L-DOPA increased the number of adjusting steps lesion rats made with both the ipsilateral and contralateral forelimb, with no significant difference observed between the two strains (Drug: $F_{1,23}=70.70$, $p<0.001$; Drug x Side: $F_{1,23}=0.33$, n.s.; Drug x Strain: $F_{1,23}=0.07$, n.s.).

Sensorimotor function was tested using the vibrissae test. While lesion rats in both strains exhibited a contralateral deficit in the test (Figure 3.4C; Side: $F_{1,23}=47.85$, $p<0.001$), this deficit was significantly more pronounced in the Sprague Dawley than in the Lister Hooded cohort (Side x Strain: $F_{1,23}=6.19$, $p<0.05$; Simple Effect of Strain on Side: $F_{1,23}=5.11$, $p<0.05$). Administration of 1 mg/kg acute L-DOPA significantly increased the number of contralateral responses made by both strains (Side x Drug: $F_{1,23}=10.51$, $p<0.05$; Simple Effect of Drug on Contralateral Side: $F_{1,23}=47.08$, $p<0.001$). The overall magnitude of the L-DOPA induced improvement was significantly greater in the Sprague Dawley than in the Lister Hooded cohort. However, while there was a trend towards a greater response exclusively on the contralateral side this did not reach statistical significance (Strain x Drug: $F_{1,23}=6.19$, $p<0.05$; Simple Effect of Strain on L-DOPA condition: $F_{1,23}=5.63$, $p<0.05$; Strain x Drug x Side: $F_{1,23}=3.67$, $p=0.067$). Visual inspection of the data together with the statistically significant strain difference during the saline condition suggested that the overall difference between the effect of L-DOPA in the two strains may have been driven by a more pronounced contralateral baseline deficit in the Sprague Dawley cohort (Figure 3.4C).

3.4.3.4. Simple discrimination task

Whilst the unilateral lesion was shown to induce contralateral impairments in hand-tests of simple motor function, there was no overall effect of the lesion on contralateral accuracy in the simple discrimination task (Figure 3.5A-B; Lesion: $F_{1,35}=1.71$, n.s.). However, relative to intact rats, lesion rats were more accurate on the second day of ipsilateral CS testing (Day x Side x Lesion: $F_{8,280}=3.17$, $p<0.05$; Simple Effect of Lesion on Day: $F_{1,35}=7.72$, $p<0.001$).

While there was no overall difference between the strains (Strain: $F_{1,35}=2.93$, n.s.), the performance of Lister Hooded rats differed significantly from that of Sprague Dawley rats on days 1 and 3 of each side of testing (Day x Strain x Lesion: $F_{8,280}=2.21$, $p<0.05$; Simple Effect: min. $F_{1,35}=4.87$, $p<0.05$). However, while Lister Hooded rats were significantly more accurate on the first days of contralateral and ipsilateral testing, and had a significantly overall lower performance than Sprague Dawley rats on the third day of contralateral and ipsilateral testing it should be noted that there were a lot of noise in the data which limits the conclusions that can be drawn from this statistical significance.

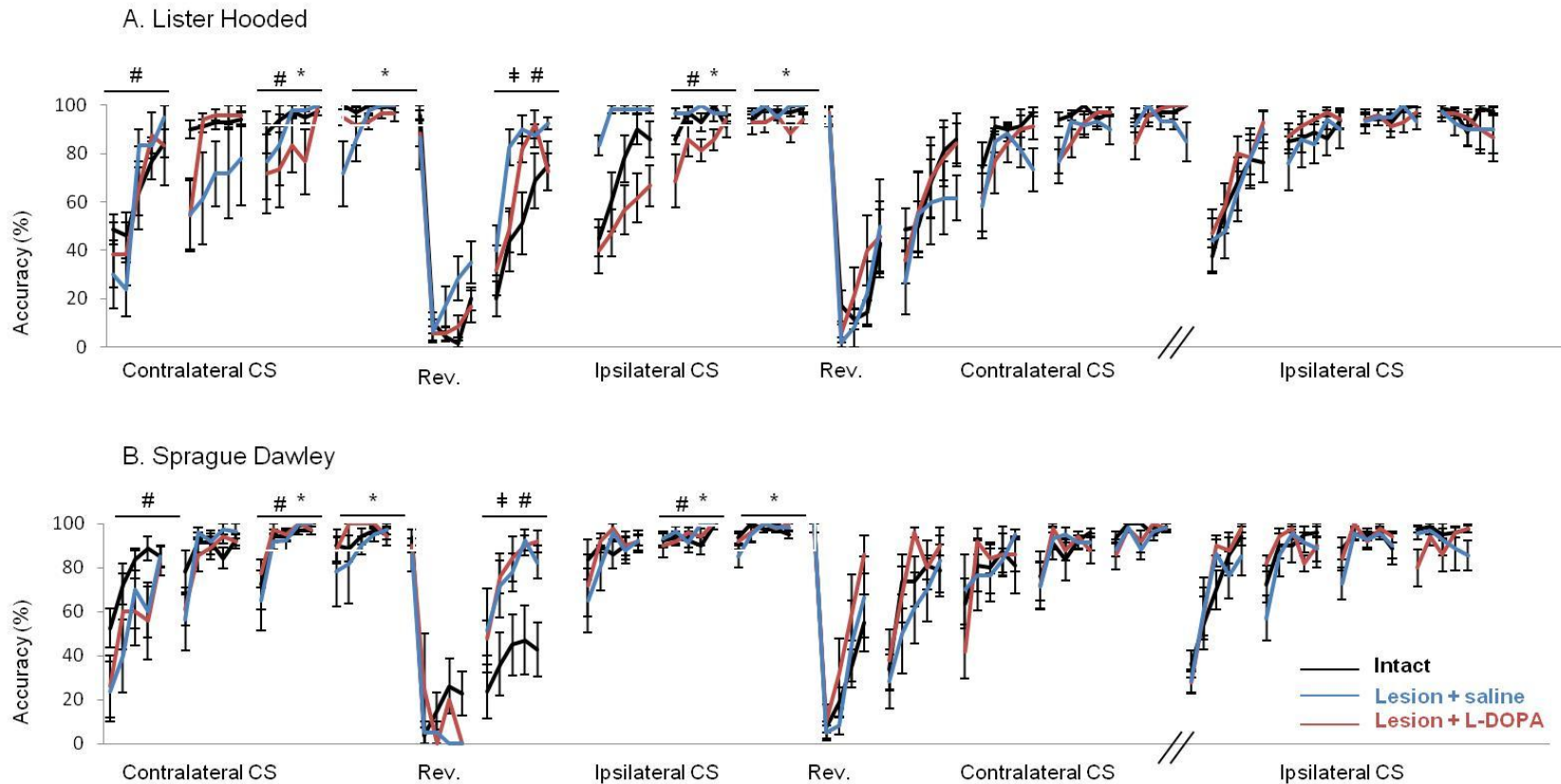


Figure 3.5. The performance of Lister Hooded (A) and Sprague Dawley (B) rats on a simple discrimination task where half the lesion rats were administered 1 mg/kg L-DOPA prior to testing while the remaining rats were administered 1 ml/kg saline. Each continuous line represents one day's performance, broken down into 5 blocks of 10 trials each. In total, rats were tested over 18 days. Half of these days, rats were rewarded for pressing the lever contralateral to the lesion and the remaining half rats were rewarded for pressing the lever ipsilateral to the lever (labelled 'Contralateral CS' and 'Ipsilateral CS', respectively, on the x-axis). The line charts show the mean and the error bars show the standard error of the mean. CS=Conditioned stimulus, Rev.= Reversal to ipsilateral/contralateral CS.

*** Effect of L-DOPA, † Effect of Lesion, # Effect of Strain. *p<0.05, † p<0.05, # p<0.05**

Performance between blocks (i.e. sets of ten consecutive trials) was analysed to provide a measurement of learning occurring within daily testing sessions. All rats' performance improved within each daily testing session (Figure 3.5; Block: $F_{4,140}=89.51$, $p<0.001$). Lesion rats performance differed significantly from that of intact rats in the first block of testing (Block x Lesion: $F_{4,140}=6.87$, $p<0.01$; Simple Effect of Lesion on Block: $F_{1,35}=6.87$, $p<0.05$). Whilst Lister Hooded rats tended to show overall greater within-session learning than Sprague Dawley rats (Block x Strain: $F_{4,140}=4.62$, $p<0.01$), there was no difference in the effect which a lesion had on within-session learning in the two strains (Block x Strain x Lesion: $F_{4,140}=0.48$, n.s.).

Acute L-DOPA administration did not induce an overall improvement in accuracy (Figure 3.5; Drug: $F_{1,35}=1.66$, n.s.), nor was there a difference in the effects of acute L-DOPA administration on the two strains (Side x Strain x Drug: $F_{1,35}=1.66$, n.s.; Strain x Drug: $F_{1,35}=0.16$, n.s.), or an effect of acute L-DOPA on within-session performance (Block x Drug: $F_{4,140}=0.75$, n.s.; Block x Strain x Drug: $F_{4,140}=0.83$, n.s.). L-DOPA did, however, mildly impair performance on the third and fourth day of testing (Day x Drug: $F_{8,280}=3.12$, $p<0.01$; Simple Effect of Drug on Day: $F_{1,35}=6.63$, $p<0.05$; Side x Day x Drug: $F_{8,280}=1.37$, n.s.).

Sucrose Pellet Consumption Tests. Two versions of the sucrose consumption test, using a 0 s and 5 s ITI respectively, were conducted to assess rats' motivation to consume sucrose pellets in the same operant boxes that had been used in the simple discrimination task. All rats consumed more pellets in the 0 s ITI, than the 5 s ITI, version of the task (Figure 3.6; ITI: $F_{1,35}=98.95$, $p<0.001$). There was no difference in the number of sucrose pellets consumed by Lister Hooded and Sprague Dawley rats (Strain: $F_{1,35}=0.02$, n.s.; ITI x Strain: $F_{1,35}=0.30$, n.s.), nor between lesion and intact rats (Lesion: $F_{1,35}=2.79$, n.s.; ITI x Lesion: $F_{1,35}=0.21$, n.s.), or rats administered acute L-DOPA or saline prior to testing (Drug: $F_{1,35}=0.64$, n.s.; ITI x Drug: $F_{1,35}=0.01$, n.s.).

3.2.4. Discussion

The primary aim of the experiment was to determine whether (i) Lister Hooded rats could be used in the place of Sprague Dawley rats in L-DOPA and dyskinesia experiments, and (ii) whether lesion rats that had received chronic L-DOPA treatment

could be tested in operant chambers following acute administration of a low dose of acute L-DOPA without the presence of disabling dyskinesias. To this end, lesion rats from the two strains were chronically treated with L-DOPA and compared in their AIMs and motor response to a low dose of acute L-DOPA. In addition, their performance on a simple discrimination and reversal learning task following acute administration of either L-DOPA or saline was compared. Based on the data

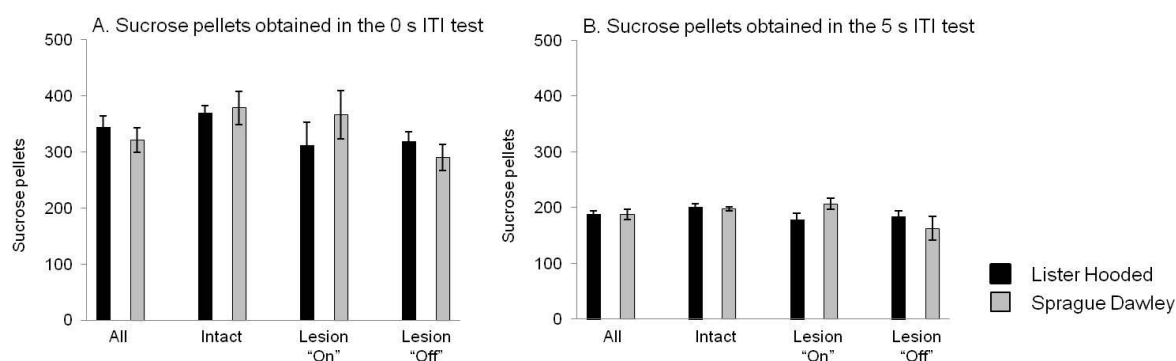


Figure 3.6. The mean number of sucrose pellets obtained by Lister Hooded (n=21) and Sprague Dawley (n=20) rats on a sucrose pellet consumption test allowing rats to panel press for new pellets with a 0 s (A) or 5 s (B) ITI. "On" and "off" refer to lesion rats administered 1 mg/kg acute L-DOPA ("on") or 1 ml/kg saline ("off") prior to testing. The error bars show the standard error of the mean. ITI=Intertrial interval.

presented in this section, it was concluded that (i) lesion Lister Hooded rats could be used in dyskinesia experiments, and (ii) that it was possible to administer a low L-DOPA dose to lesion rats that had previously received chronic L-DOPA treatment testing in operant chambers without inducing AIMs of such magnitude so as to interfere with operant performance.

Following chronic L-DOPA treatment, acute administration of 10 or 1 mg/kg L-DOPA induced AIMs in lesion rats of both strains. Interestingly, Lister Hooded rats exhibited more pronounced AIMs than Sprague Dawley rats when administered acute L-DOPA post chronic treatment. The onset of LID and expression of AIMs have both been linked to sensitisation and activation of D1 type receptors (Aubert *et al*, 2005; Mela *et al*, 2012). Previous studies have suggested that rat strains differ in regard to their dopamine receptor and dopamine transporter binding (Zamudio *et al*, 2005). While strain differences between Lister Hooded and Sprague Dawley rats' D1 receptors have not, to my knowledge, been specifically explored, Lister Hooded rats

show a greater locomotor response to amphetamine than Sprague Dawley rats, a phenomenon which has been speculated to be due to differences in the two strains' dopamine receptor sensitivity (McDermott & Kelly, 2008). Hence, whereas it was beyond the scope of the current experiment to determine the reasons for the strain difference in AIMs scores, it may be speculated that it was due to differences in Lister Hooded and Sprague Dawley rats' D1 receptor sensitivity.

A primary aim of the experiment was to determine whether Lister Hooded rats could be used in L-DOPA and dyskinesia experiments instead of Sprague Dawley rats, which are more common in such studies (e.g. Monville *et al*, 2005; Breger *et al*, 2013). Although the strains differed in the magnitude of their AIMs scores, LID was successfully induced in all lesion L-DOPA treated rats. Previous studies on rats and humans have shown between-species consistency in the mechanisms underlying LID (e.g. Andersson *et al*, 1999; Lindgren *et al* 2011; Verhagen *et al*, 1998; Breger *et al*, 2013). Thus, while there were strain differences in the magnitude of AIMs expression these are unlikely to reflect differences in the mechanisms underlying LID and AIMs in Lister Hooded versus Sprague Dawley rats. For this reason, and because Lister Hooded rats are preferred in operant studies where high visual acuity is necessary, it was considered valid to use Lister Hooded rats in lieu of Sprague Dawley rats in later experiments where LID onset and operant behaviour were explored simultaneously.

Because later experiments would administer acute L-DOPA to lesion rats who had developed LID prior to operant testing, the current experiment also tested whether a low dose of acute L-DOPA could improve motor function in lesion rats that had developed LID without simultaneously causing dyskinesias of such magnitude as to interfere with operant performance. In line with previous data (e.g. Lindgren *et al*, 2007) it was demonstrated that decreasing the acute L-DOPA dose from 10 mg/kg to 1 mg/kg reduced AIMs magnitude without completely abolishing the dyskinetic response to L-DOPA; suggesting a modest yet still measurable motor effect of the lower dose.

However, while it has previously been shown that acute L-DOPA doses higher than that used here improve 6-OHDA lesion rats' performance on the adjusting step test (8 mg/kg; Olsson *et al*, 1995) the current data did not show improved forelimb function following administration of 1 mg/kg L-DOPA. Neither did it show an effect of acute L-DOPA on spontaneous activity as measured by the number of beam breaks recorded in activity chambers. The latter could be caused by limitations to the

apparatus used as the activity cages used were designed for mice and not rats (width at bottom of cage=20 cm; length at bottom of cage=36 cm). There was, however, improvement on the vibrissae test which, together with the low but measurable dyskinetic response to 1 mg/kg L-DOPA, suggested that the dose was sufficient to induce a motor response with minimal observable AIMs. Based on the data, the lower dose of 1 mg/kg L-DOPA dose was subsequently used in the simple discrimination task. Whilst it was not possible to conduct dyskinesia scoring during operant testing, rats were closely monitored before and after the operant task and, when possible, on video screens whilst in the operant boxes. Such observations did not reveal any instances of AIMs preventing rats' from performing the task.

In line with the hypothesis and previous findings by e.g. Robbins and colleagues (1990), data from the simple discrimination task showed impaired performance in lesion relative to intact rats, albeit subtle. However, contrary to the hypothesis, the current data only showed a marginal effect of acute L-DOPA on operant performance. This may either suggest that the task was not sensitive to the psychopharmacological effects of the drug, or that the ability for all lesion rats in the current experiment to acquire the simple discrimination rule caused a ceiling effect which limited the possibility of observing a beneficial effect of L-DOPA. It is possible that more complex operant tasks are needed in order to observe a robust effect of lesion and acute L-DOPA in rats with unilateral 6-OHDA, MFB lesions.

Following operant testing, rats underwent two versions of a sucrose consumption test, using a 0 s and a 5 s ITI respectively. The 5 s ITI version was considered less sensitive to motor impairments, as the longer ITI enabled both intact and lesion rats to complete consumption of an acquired sucrose pellet before they were able to panel press for another pellet. Therefore, motor deficits were considered less likely to affect the total number of sucrose obtained by rats in the 5 s ITI than in the 0 s ITI version of the task. The data showed that neither the lesion nor acute L-DOPA affected the total number of sucrose pellets consumed by the rats. This was true for both the 0 s and 5 s ITI version of the task. Thus, there was no evidence for an effect of lesion, acute L-DOPA, or strain on lesion rats' willingness to consume sucrose pellets in the current experiment.

To conclude, the aim of the experiment was to determine whether Lister Hooded rats could be used in experiments combining the study of operant behaviour and dyskinesia, despite dyskinesia being more commonly studied in Sprague Dawley

rats (e.g. Monville *et al*, 2005; Breger *et al*, 2013). The experiment also aimed to determine whether a low dose of acute L-DOPA could produce a motor response in lesion rats that had been chronically treated with L-DOPA without simultaneously causing disabling AIMs. Based on the described data, it was decided to (i) use Lister Hooded rats in subsequent experiments incorporating both dyskinesia and operant testing (Chapters 7-8), and (ii) use the 1 mg/kg L-DOPA dose when acute L-DOPA was administered pre-operant testing to lesion rats that had previously received chronic L-DOPA treatment in Chapter 7.

3.3. Experiment 2: Using non-pharmacological tests to determine lesion success in the unilateral 6-OHDA MFB model

3.3.1. Introduction

As described in the chapter introduction, the standard apomorphine and amphetamine induced rotations were deemed inappropriate methods for screening lesion success in later thesis experiments. To find reliable methods of assessing lesion extent that do not rely on pharmacological stimulation of the dopaminergic system, the ability of amphetamine-induced rotations and three non-invasive behavioural tests to accurately predict lesion success in male rats with unilateral 6-OHDA lesions to the MFB was compared.

The rats from which the histological and motor data were obtained had been part of an operant pilot not reported in this thesis. For the purpose of the pilot experiment the same lesion protocol had been used for all rats, rather than attempting to produce lesions of varying size as would have been done had the original purpose been to identify correlations between dopamine loss and motor behaviour. The aim of the reported analysis was to use the histological and motor data already obtained from these rats to identify motor tests that could be used to screen for rats with a SNc dopamine loss of $\geq 90\%$, which was the cut-off criterion applied in the thesis project when deciding whether to include or exclude rats in final data analyses.

Based on the results from the analysis, two of the non-invasive behavioural tests (spontaneous rotations and the cylinder test) were used when screening for lesions in later thesis experiments. At the completion of these experiments, a retrospective analysis including spontaneous rotations, cylinder test performance, and TH+ counts from later experiments was conducted to validate the initial findings.

When available, AIMs scores from later experiments were also included in the analysis to test whether these correlated with performance on either behavioural test.

3.3.1.1. Aims

The aim of the first analysis was to compare the ability of amphetamine-induced rotations, spontaneous rotations, the elevated body swing, and the cylinder test to identify rats with SNc dopamine denervation $\geq 90\%$. The experiment used male Lister Hooded rats with unilateral 6-OHDA lesion to the MFB.

A second analysis was thereafter conducted in which behavioural and histological data from female Lister Hooded rats used in Chapters 6-8 were included. The aim of this was both to replicate the initial findings and to test whether spontaneous rotations or the cylinder test correlated with, or could predict, the magnitude of later AIMs.

3.3.2. Methods

3.3.2.1. Experimental Design

Analysis of male Lister Hooded rats. Rats were given unilateral lesions to the MFB. Following 14 days recovery, their performance on the cylinder test, the elevated body swing and the net bias of their spontaneous rotations were measured. Following brief operant testing as part of an operant pilot experiment (data not included in this thesis) that utilised the simple discrimination task (2.7.1.2), the rats' amphetamine-induced rotations were recorded. The rats were then perfused and the brain tissue taken for histological analysis (Figure 3.7).

It should be noted that (i) male rats were chosen instead of female rats used because the cohort was originally ordered to replicate data obtained using male rats, and (ii) because the original intended use of the rats had not been a lesion screening/correlation experiment all lesions were conducted using the same lesion protocol. Had the original intention been to conduct a correlation/lesion screening experiment, the experiment would have been designed to produce lesions of varying size.

Analysis of female Lister Hooded rats. Rats included in the second analysis had undergone varied testing and pharmacological treatments prior to culling, as outlined in Chapters 6-8. In all instances, rats had been given unilateral 6-OHDA MFB lesions, screened using the cylinder test and spontaneous rotations, and been tested on the

staircase and LCRT tasks. In some instances rats had undergone chronic L-DOPA treatment after testing on the cylinder and spontaneous rotations tests.

| | FR & Operant training | Lesion | Recovery | Elevated body swing | Spont. rotations | Cylinder test | FR | Operant testing | Amphet. Rotations | Perfusion |
|-------------------------------|--|--------------------------------|-----------------------|--------------------------|--|--|------------------|--|---|-----------|
| Duration (days) | 25 | 2 | 14 | 1 | 1 | 1 | 7 | 25 | 1 | 1 |
| Cumulative time (days) | 25 | 27 | 41 | 42 | 43 | 44 | 51 | 76 | 77 | 78 |
| Details | Food restriction and lever press training in two lever operant boxes | Unilateral, 6-OHDA MFB lesions | Recovery from lesions | Elevated body swing test | Recording of rotational bias in rotometers in the absence of drugs | Recording ipsilateral bias of the first 10 paw placements made in a plexiglas cylinder | Food restriction | Pilot study requiring lever pressing for sucrose pellets. <i>Ad libitum</i> access to food at end of the operant testing block | Recording rotational bias in rotometers following 2.5 mg/kg amphet. | Perfusion |

Figure 3.7. A graphical representation of the experimental design used in the lesion screening experiment including male Lister Hooded rats. The figure specifies both the duration of the individual testing phases, and the overall number of days in the experiment. Light grey boxes indicate testing not reported in this thesis. Amphet.=Amphetamine, FR=Food restriction, MFB=Medial forebrain bundle, Spont. rotations = Spontaneous rotations.

3.3.3.2. Procedure

Analysis of male Lister Hooded rats. Twenty-one male Lister Hooded rats were given unilateral 6-OHDA lesions to the MFB (section 2.2.2). Following 14 days recovery their motor impairment was measured using the elevated body swing (section 2.4.5), spontaneous rotations (section 2.4.6.3) and the cylinder test (section 2.4.4). After 25 days of participation in a pilot study (not reported), the rats were tested for amphetamine-induced rotations as described in section 2.4.6.2. The amphetamine-induced rotations were conducted at the end of testing to avoid the possibility of amphetamine exposure affecting performance on the other tests.

The non-invasive motor tests all measured the presence of a directional motor bias following a unilateral lesion. The elevated body swing measures the direction which rats turn their bodies when lifted by the base of their tail (Borlongan & Sanberg, 1995), the spontaneous rotation test measures the ipsilateral locomotor behaviour lesion rats exhibit when placed in a circular environment, and the cylinder test measures rats' preference for using their ipsilateral over their contralateral forelimb during explorative behaviour in a Plexiglas cylinder (Schallert *et al*, 2000). Previous publications have demonstrated that unilateral lesions induce a spontaneous

ipsilateral bias on the elevated body swing (Borlogan & Sanberg, 1995), an ipsilateral bias in non-drug induced locomotive behaviour (Ungerstedt, 1968; Fornaguera *et al*, 1994), and a preference for using the ipsilateral over the contralateral paw in the cylinder task (Schallert *et al*, 2000). Amphetamine-induced rotations, which are more commonly used, measure the rotational bias observed following acute administration of amphetamine. Previous publications have reported an enhancement of the spontaneous ipsilateral bias exhibited by rats with unilateral 6-OHDA lesions following acute amphetamine administration (Ungerstedt & Arbuthnott, 1970; Torres *et al*, 2010).

In the operant pilot study (not reported), rats were food restricted and underwent testing on the simple discrimination task described in sections 2.7.1.2 and Experiment 1. No drugs were administered as part of the pilot experiment and the rats were not tested on other motor behavioural tests than those reported in the results section. Once all operant and behavioural testing was completed, rats were euthanized by an overdose of Euthatal, perfused (section 2.8) and their brain tissue was taken for TH immunohistological staining (section 2.11).

Analysis of female Lister Hooded rats. Rats included in the retrospective analysis had undergone varied behavioural testing prior to culling as further detailed in Chapters 6-8. A total of 169 lesion female Lister Hooded rats were included. All rats had undergone unilateral 6-OHDA MFB lesion (section 2.2.2) and, following 14 days recovery from surgery, been screened for lesions using the spontaneous rotation (section 2.4.6.3) and cylinder (section 2.4.4) tests. The correlation between the rats' SNc dopamine loss, VTA dopamine loss, ipsilateral bias on the cylinder test, and net ipsilateral bias on the spontaneous rotations were was calculated to validate the findings from the male cohort.

In addition to validating the findings obtained from the male cohort, the data from the female rats were also used to test whether the cylinder or spontaneous rotation tests could predict later AIMs scores. Following lesion screening, 53 of the 169 rats had received chronic L-DOPA treatment consisting of daily injections of 10 mg/kg L-DOPA until their cohort exhibited stable AIMs. Note that rats that had been co-treated with a TAT2A peptide in an attempt to prevent LID onset in Experiment 10 were not included in this analysis. A second correlation analysis only including the 53 lesion, L-DOPA treated rats was conducted. The analysis contained the same

variables as above, as well as the cumulative AIMs score obtained from the rats' final AIMs scoring session.

3.3.3.3. Statistical analysis

For all analyses, cell counts of dopaminergic cell loss in the SNc and VTA were expressed as a percentage of the counts on the intact side of the brain. In the male cohort, the loss of dopaminergic cell bodies in the ipsilateral SNc and VTA, the ipsilateral bias on the cylinder and elevated body swing tests were not normally distributed. As parametric tests are more robust than their non-parametric counterparts, the data from the surviving SNc and VTA neurons was transformed using the Lg10 values, which resulted in them showing of a normal distribution. As the data from the cylinder and elevated body swing could not be transformed successfully the original values from these tests were used in the analysis. Dopamine loss was correlated with the parametric rotational bias using a Person correlation. All other correlations were conducted using a Spearman rho correlation. Finally, a partial correlation analysis was conducted. In addition to the correlations, one-way ANOVAs (for normally distributed data) or Mann-Whitney U tests (for non-normally distributed data) tests were conducted to determine whether there was a significant difference in motor performance between rats with $\geq 90\%$ and $< 90\%$ TH loss in the lesion SNc.

In the analysis including female Lister Hooded rats, only the spontaneous rotation data showed a normal distribution. As the remaining data could not be transformed into a normal distribution all correlation data was correlated using a Spearman rho correlation. Between group differences were analysed using a Kruskal Wallis (in the case of spontaneous rotations) or a one-way ANOVA (in the case of the cylinder test).

3.3.3. Results

3.3.3.1. Analysis of Male Lister Hooded rats

Dopamine depletion. The percentage of remaining dopaminergic cells ranged from 2% to 72% in the lesion SNc, and from 9% to 86% in the lesion VTA (Figure 3.8A). The median percentage of surviving TH+ cells was 8% (1st and 3rd quartile: 4% and 19%) in the SNc and 22% (1st and 3rd quartile: 15% and 40%) in the VTA. Nine of the 21 rats showed less than 90% SNc depletion, which was the cut-off line used in other experiments to distinguish between lesion and non-lesion rats. The spread in the

dopamine denervation, which was greater than in later thesis experiments, made it appropriate to analyze the data using both correlations and cut-off thresholds.

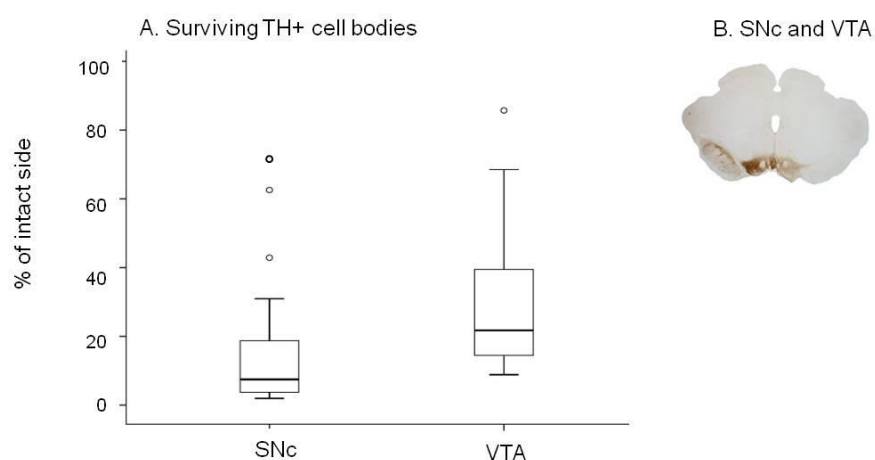


Figure 3.8. The median number of surviving TH+ cell bodies in the ipsilateral SNc and VTA, expressed as a percentage of the intact side (A), together with a photo of the SNc and the VTA (B) of a lesion rat included in the experiment. The boxplot shows the median, and the interquartile ranges, with the circles representing outliers. SNc=Substantia nigra pars compacta, TH=Tyrosine hydroxylase, VTA=Ventral Tegmental Area.

Relationship between motor behaviour and SNc dopamine loss. The rats exhibited an ipsilateral bias on all four behavioural tests (Figure 3.9). The relationship between dopamine loss and ipsilateral bias was explored both using correlations and by exploring what, if any, thresholds that could be applied to the motor data to distinguish between rats with $\geq 90\%$ and $< 90\%$ SNc dopamine loss.

SNc denervation correlated significantly with the ipsilateral bias exhibited in the cylinder test (Figure 3.10A; $r=0.70$, $n=21$, $p<0.01$), the spontaneous rotations (Figure 3.10B; $r=0.64$, $n=21$, $p<0.01$), and the amphetamine-induced rotations (Figure 3.10C; $r=0.52$, $n=21$, $p<0.05$), but not with the net ipsilateral bias on the elevated body swing test (Figure 3.10D; $r=0.32$, $n=21$, n.s.). A further analysis was conducted to determine whether there was a statistically significant difference in the performance of rats with $\geq 90\%$ or $< 90\%$ TH loss in the SNc. A significant difference between the groups was observed in the spontaneous rotation ($F_{1,20}=5.85$, $p<0.05$) and cylinder tests ($U=9$, $n=21$, $p<0.05$). Conversely, the bias exhibited in the amphetamine-induced ($F_{1,20}=1.28$, n.s.) rotations and the elevated body swing

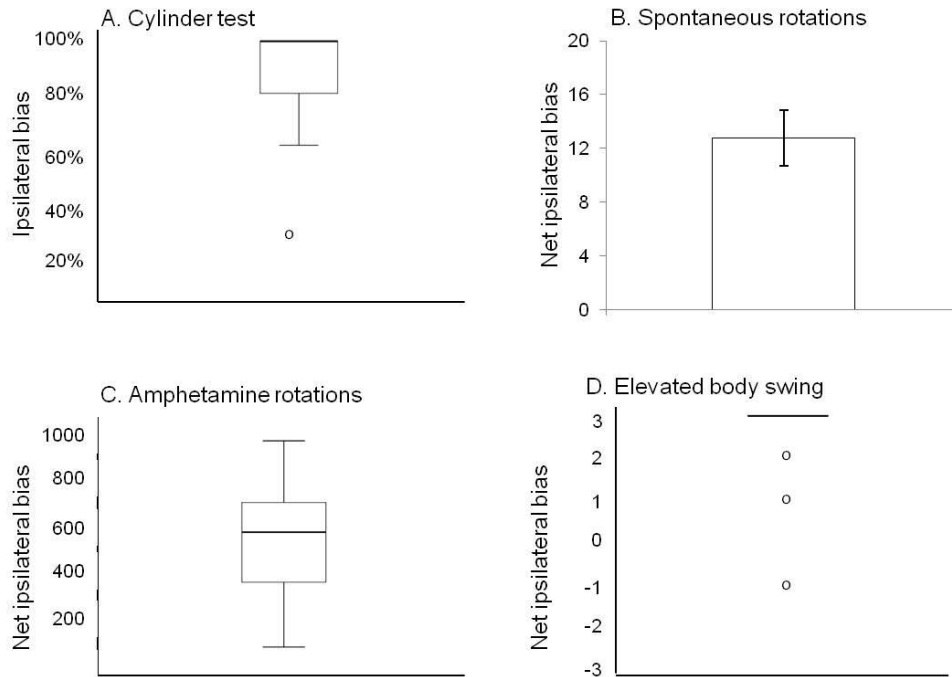


Figure 3.9. Boxplots (A, C, D) and barchart (B) illustrating the median (A, C, D) and mean (B) ipsilateral bias on the cylinder test (A), spontaneous rotations (B), amphetamine-induced rotations (C), and elevated body swing (D) tests in male Lister Hooded rats with unilateral 6-OHDA lesions to the MFB. The boxplots (A, C, D) show the median and interquartile ranges, with circles representing outliers. The error bars in the bar chart (B) show the standard error of the mean.

($U=40.50$, $n=21$, n.s.) did not differ between rats with a dopamine denervation of $<90\%$ and $\geq 90\%$.

Data from the cylinder and spontaneous rotations tests, where a significant difference between rats with $\geq 90\%$ and $<90\%$ SNc dopamine loss was observed, were further studied to determine appropriate cut-off thresholds for identifying rats with $\geq 90\%$ SNc denervation. By using a 100% ipsilateral bias on the cylinder test as a cut-off line, 83.3% of rats with a SNc denervation of $\geq 90\%$ were identified and only 11.1%, i.e. a single rat, of the group with $<90\%$ SNc dopamine loss was falsely identified. The falsely identified rat had a SNc dopamine loss of 83%. For the spontaneous rotations, using an ipsilateral net bias of ≥ 10.0 also identified 83.3% of all rats with a SNc dopamine denervation $\geq 90\%$, but falsely identified 33.3% of the rats with lesser lesions. The average SNc denervation of the falsely identified rats was 82% (sem $\pm 10\%$).

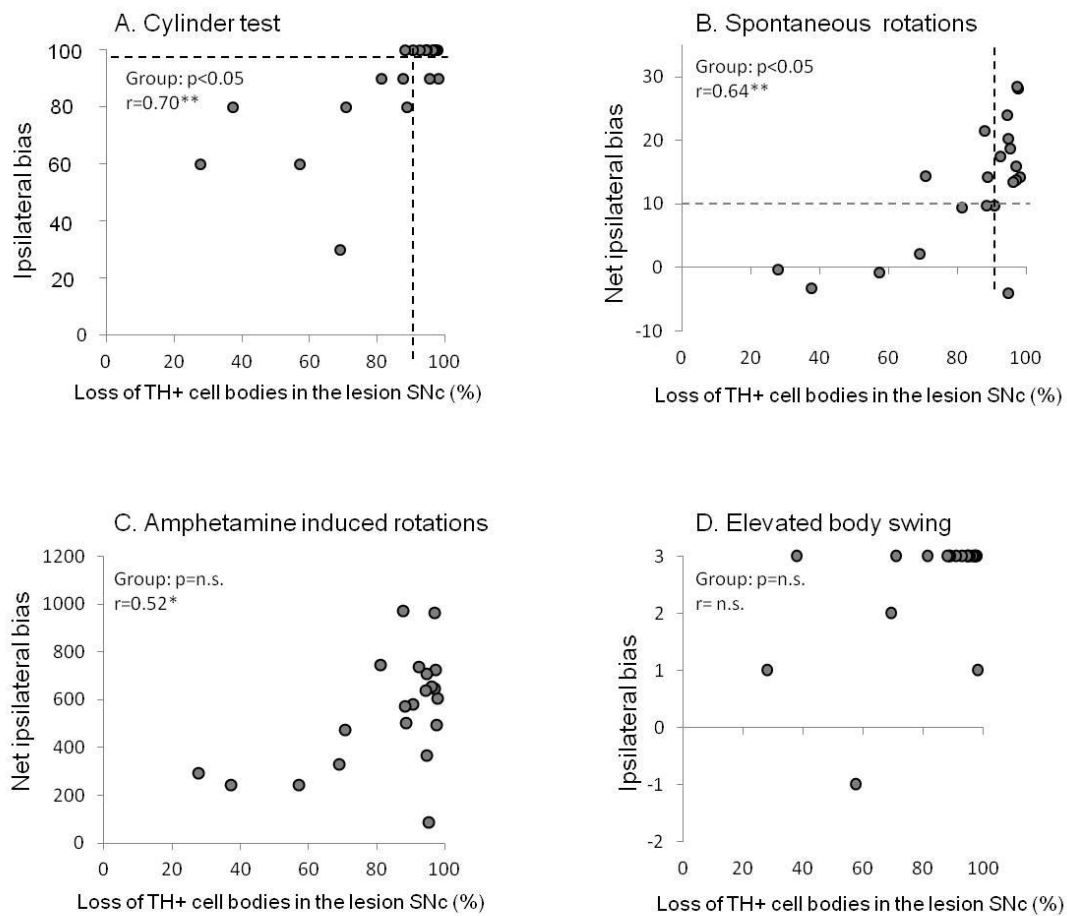


Figure 3.10. Scatter plots showing the relationship between percentage dopaminergic cell loss in the ipsilateral SNc and performance on the cylinder test (A), spontaneous rotations (B), amphetamine-induced rotations (C) and the elevated body swing (D) in 21 male rats with unilateral lesions to the MFB. For each scatterplot, it is specified whether there was a significant difference in the performance of rats with <90% and $\geq 90\%$ SNc dopamine loss, and whether there was a significant correlation between dopamine loss and performance. The dotted line in graphs A and B show the artificial cut-off line that was most appropriate for determining lesion success. n.s.=not significant; SNc=Substantia nigra pars compacta; TH=Tyrosine hydroxylase. * $p < 0.05$, ** $p < 0.01$.

Correlation between motor behaviour and VTA dopamine loss. The purpose of the experiment was to identify behavioural tests that could identify rats with $\geq 90\%$ TH loss in the SNc. Because experiments reported in this thesis did not excluded rats based on VTA denervation, it was not appropriate to determine cut-off thresholds to identify rats with large dopamine loss in the VTA. However, because the data showed

a significant correlation between loss of SNc and loss of VTA dopaminergic neurons ($r=0.61$, $n=21$, $p<0.01$), non-parametric Spearman correlations were calculated to test whether there was a relationship between dopaminergic depletion in the VTA and performance on the behavioural tests (Figure 3.11). The analysis showed a significant correlation between net spontaneous rotations and VTA denervation ($r=0.62$, $n=21$, $p<0.01$) whilst net amphetamine-induced rotations ($r=0.40$, $n=21$, n.s.), the cylinder test ($r=0.36$, $n=21$, n.s.) and the elevated body swing ($r=0.13$, $n=21$, n.s.) tests did not correlate with dopamine loss in the VTA.

Partial correlations. The data showed a significant correlation between SNc and VTA loss ($r=0.61$, $n=21$, $p<0.01$). To determine the impact of denervation in each region on the ipsilateral bias exhibited on the behavioural tests a partial correlation analysis was conducted. When controlling for SNc loss, VTA denervation no longer correlated significantly with any of the behavioural tests. Conversely, when controlling for VTA depletion, dopaminergic loss in the SNc still correlated significantly with ipsilateral bias on the cylinder test, albeit with a lower correlation coefficient ($r=0.56$, $n=21$, $p<0.05$) whilst the correlation with the other behavioural tests lost significance. This suggests that only performance on the cylinder test was dependent on SNc, but not VTA, denervation.

Correlations with amphetamine-induced rotations. Amphetamine-induced rotations are commonly used to screen lesion success in the unilateral 6-OHDA rat model. Therefore, a correlation between the net bias on this test and performance on the other behavioural tests was also conducted. Although the correlation coefficients were not high, amphetamine-induced rotations correlated significantly with ipsilateral bias on the cylinder test (Table 3.1; $r=0.55$, $n=21$, $p<0.01$), net spontaneous rotations ($r=0.53$, $n=21$, $p<0.05$), and the elevated body swing ($r=0.43$, $n=21$, $p<0.01$).

3.3.3.2. Analysis including Female Lister Hooded rats

Based on the data obtained from the male Lister Hooded rats, described above, the cylinder and spontaneous rotations tests were used to balance experimental groups following lesion in Chapters 6-8. At the end of these experiments, the histological and motor data obtained in these experiments were used to retrospectively validate the earlier findings.

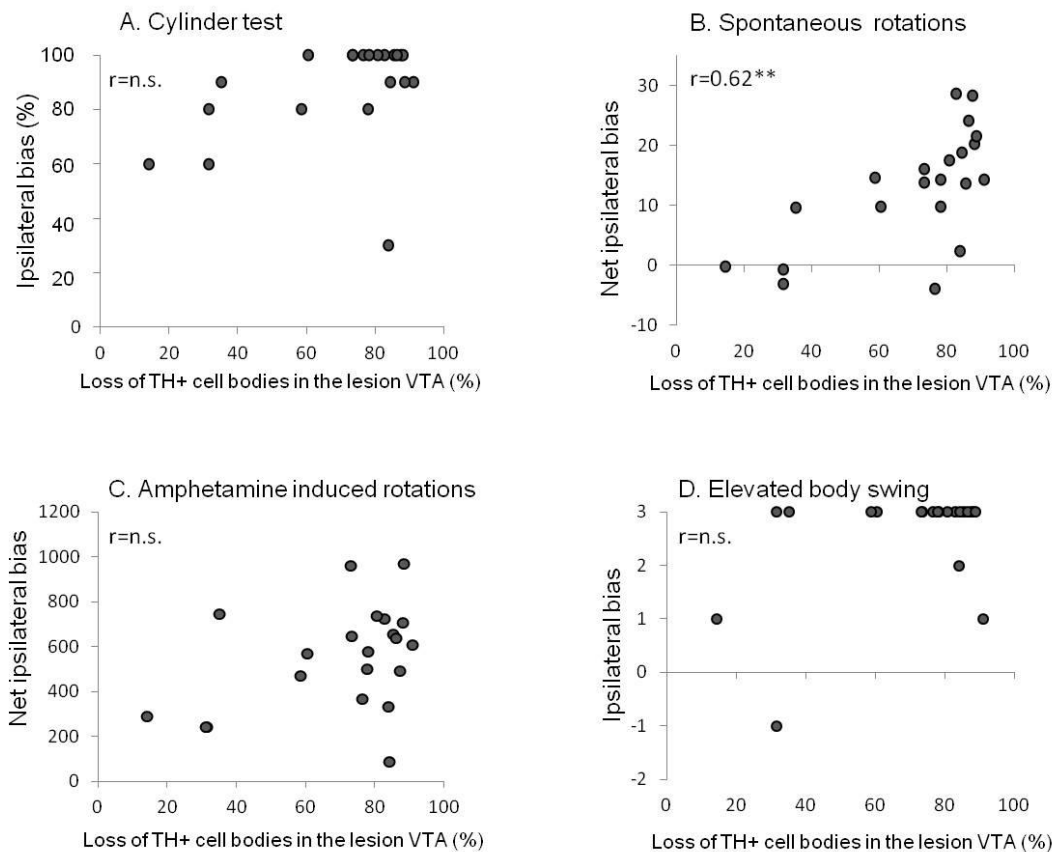


Figure 3.11. Scatter plots showing the relationship between the percentage dopaminergic cell loss in the VTA and performance on the cylinder test (A), spontaneous rotations (B), amphetamine-induced rotations (C) and the elevated body swing (D) in 21 male rats with unilateral lesions to the MFB. For each scatterplot, it is specified whether there was a significant correlation between VTA dopamine loss and performance. n.s.=not significant; TH=Tyrosine hydroxylase; VTA=Ventral tegmental area. **p<0.01.

Table 3.1. The correlations between the ipsilateral bias exhibited by 21 male Lister Hooded rats' amphetamine-induced rotations (Amphet. Rot.), spontaneous rotations (Spont.rot.), the cylinder test and the elevated body swing. n.s.=not significant. *p<0.05, **p<0.01

| | Spont. Rot. | Cylinder Test | Body Swing |
|---------------|-------------|---------------|------------|
| Amphet. Rot. | 0.53* | 0.55** | 0.43* |
| Spont. Rot. | | 0.43* | n.s. |
| Cylinder Test | | | 0.64** |

Dopamine depletion. In the initial analysis, including all 169 female rats, the average loss of dopaminergic cell bodies in the SNc and VTA was 94% (Figure 3.12A; sem $\pm 17\%$) and 84% (sem $\pm 22\%$) respectively. Out of the 169 rats, only 19 rats showed a SNc dopamine loss $<90\%$. All rats showed an ipsilateral bias on the cylinder test (Figure 3.12B) and in their spontaneous rotations (Figure 3.12C).

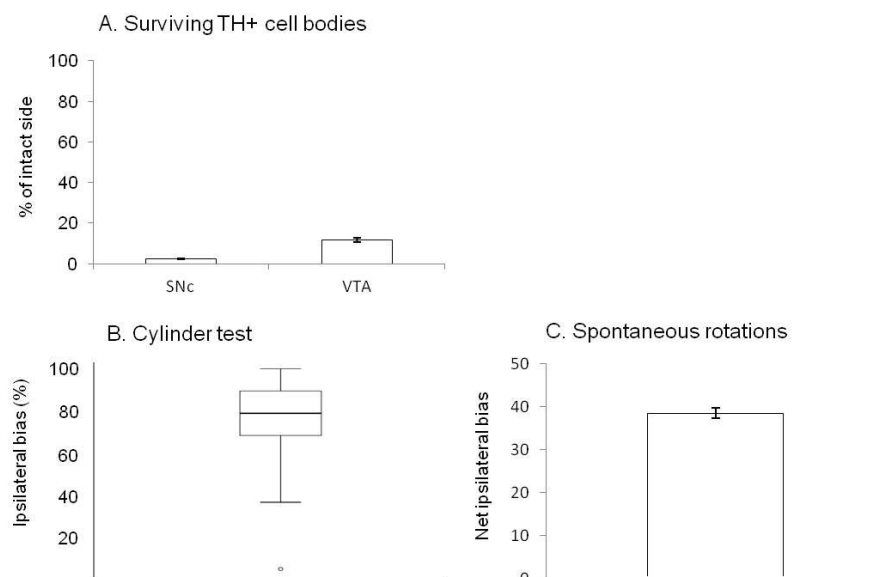


Figure 3.12. Loss of TH+ cell bodies in the ipsilateral SNc and VTA, expressed as a percentage of the intact side (A), of the 169 female Lister Hooded rats included in the second lesion screening analysis, together with their ipsilateral bias on the cylinder test (B) and spontaneous rotations test (C). The bars charts (A and C) show the mean and the standard error of the mean. The boxplot (B) shows the median and interquartile ranges, with circles representing outliers. SNc=Substantia nigra pars compacta; TH=tyrosine hydroxylase; VTA=Ventral tegmental area.

In the second analysis, which only included the 53 rats that had received chronic L-DOPA treatment post lesion and post lesion screening, the average loss of dopaminergic cell bodies in the SNc and VTA was 95% (sem $\pm 2\%$) and 85% (sem $\pm 3\%$) respectively. Out of these 53 rats, only 3 rats showed a SNc dopamine loss $<90\%$.

Relationship between motor behaviour and SNc dopamine loss. An analysis including all female rats (n=169) was conducted to test the ability of the spontaneous rotations and cylinder tests to predict lesion extent or distinguish between rats with

$\geq 90\%$ or $< 90\%$ dopamine loss in the SNc. An ipsilateral bias was observed in both the motor tests (Figure 3.12 B-C).

In the cylinder test, rats with a SNc dopamine denervation $\geq 90\%$ showed a median ipsilateral bias of 80% (1st quartile=70%, 3rd quartile=90%) whereas rats with a SNc dopamine denervation of $< 90\%$ showed a median ipsilateral bias of 70% (1st quartile=60%, 3rd quartile=80%) - a difference which was statistically significant (Figure 3.13A; $H(1)=5.72$, $p<0.05$). In addition there was a significant, albeit very low, correlation ($r=0.16$, $p<0.05$) between SNc dopamine loss and ipsilateral bias on the cylinder test. The data were also used to test appropriate thresholds for determination of lesion success. Applying the cut-off criterion of 100% ipsilateral bias on the cylinder test (which had been determined most appropriate in the analysis of the male Lister Hooded rats) only identified 6.7% of the rats with SNc dopamine loss $\geq 90\%$ and falsely identified 5.2% of rats showing $< 90\%$ SNc dopamine loss. None of the cut off lines that were applied to the data set during analysis could reliably identify the majority of rats with a SNc dopamine loss $\geq 90\%$ without simultaneously falsely identifying a large proportion of rats with SNc dopamine loss $< 90\%$. The most reasonable threshold was an ipsilateral bias of 80%. However, this threshold only identified 54.4% of rats with SNc dopamine depletion $\geq 90\%$ while falsely identifying 31.6% of rats with SNc dopamine loss $< 90\%$.

In the spontaneous rotations test, rats with a dopamine denervation $\geq 90\%$ had an average net ipsilateral bias of 38.6 (sem ± 1.2) whereas rats with $< 90\%$ dopamine denervation had an average ipsilateral bias of 29.6 (sem ± 3.9) - a difference which was statistically significant (Figure 3.13B; $F_{1,168}=5.90$, $p<0.05$). A correlation analysis between SNc depletion and spontaneous rotations was conducted but no significant correlation was found ($r=0.01$, n.s.). In addition, the data were used to test appropriate thresholds for determining extent of lesion. Applying the cut-off criterion that had been determined most appropriate in the analysis of male Lister Hooded rats (net ipsilateral bias of 10.0), identified 96.7% of rats with SNc dopamine loss $\geq 90\%$ but also falsely identified 94.7% of rats with a SNc dopamine loss $< 90\%$. While no clear cut off line could be obtained, the most discriminative cut off was a net ipsilateral bias of 35.0. This threshold identified 64.7% of the rats with SNc dopamine loss $\geq 90\%$ but falsely identified 42.1% of rats with a dopamine loss $< 90\%$. The average SNc dopamine loss of the falsely identified rats was 58% (sem 7%).

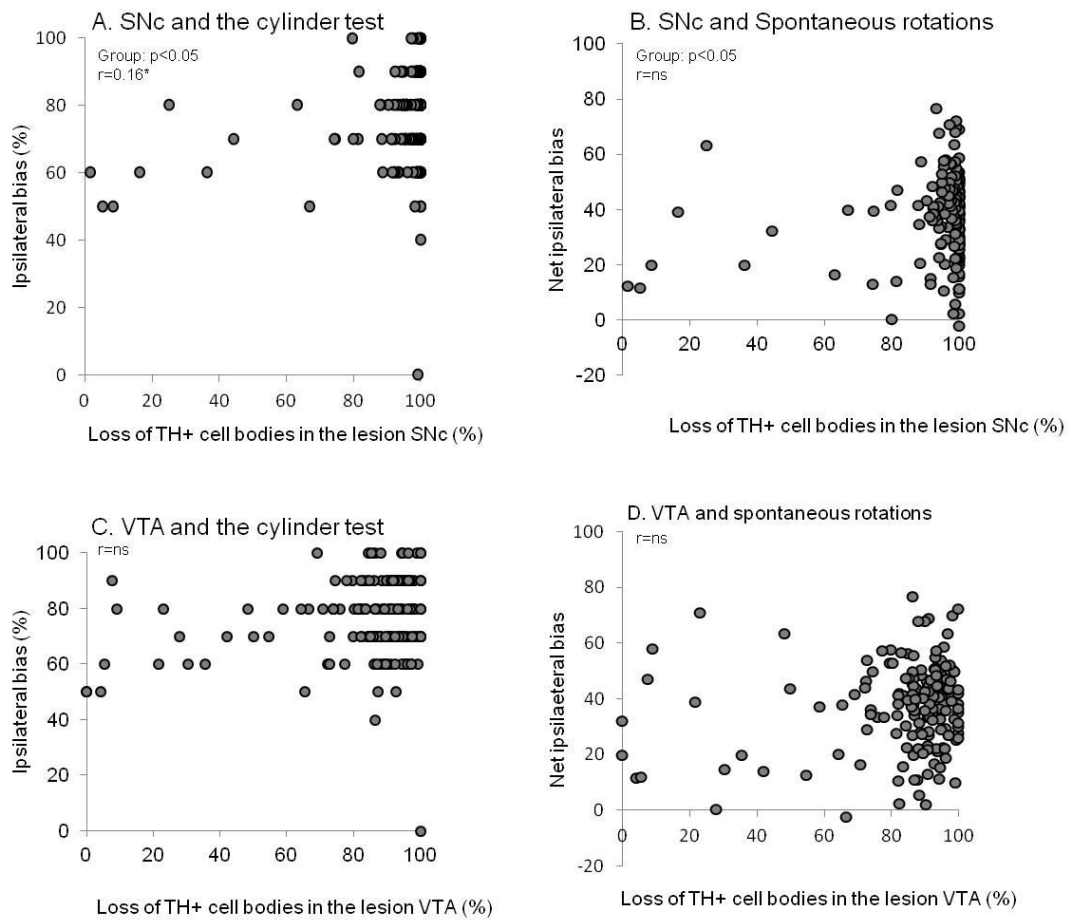


Figure 3.13. Scatter plots showing the relationship between dopaminergic cell loss in the SNc (A-B) and VTA (C-D) and ipsilateral bias on the cylinder (A, C) and the spontaneous rotations test (B, D) in 169 female rats with unilateral lesions to the MFB. The scatterplots specify whether there was a significant difference in the performance of rats with <90% and $\geq 90\%$ SNc dopamine loss on the behavioural tests (A-B), and whether there was a significant correlation between SNc/VTA dopamine loss and behavioural test performance. ns=not significant; SNc=Substantia nigra pars compacta; VTA=Ventral tegmental area. * $p < 0.05$.

Dopamine loss in the VTA did not correlate with any of the other factors included in the analysis, i.e. SNc denervation, net bias of spontaneous rotations, and ipsilateral bias on the cylinder test (Figure 3.13C-D). Because there was no correlation between VTA denervation and ipsilateral bias in the motor tests, a partial correlation was not conducted.

Correlation between motor behaviour and AIMs scores. Of the 169 female Lister Hooded rats, a subset of 53 rats had received chronic L-DOPA after lesion and testing on the cylinder and spontaneous rotations tests. An analysis only including these 53 rats was conducted in order to determine whether there was a correlation between their ipsilateral bias on the behavioural tests and subsequent AIMs scores. The median cumulative AIMs recorded from the rats over a 2 h scoring session was 35, and AIMs scores ranged from 0 to 154. There was a small but significant correlation between dopamine loss in the SNc and ipsilateral bias on the cylinder test (Table 3.2; $r=0.34$; $p<0.05$). The analysis also showed a significant, but very low, correlation between AIMs scores and the net bias of spontaneous rotations (Figure 3.14C; Table 3.2; $r=0.29$, $p<0.05$). AIMs did not correlate significantly with any of the other factors, i.e. dopamine loss in the SNc, VTA, or ipsilateral bias on the cylinder test.

Table 3.2. Correlations between loss of dopaminergic bodies in the SNc, VTA, the net ipsilateral bias of spontaneous rotations, and ipsilateral bias on the cylinder test and AIMs using collated data from 53 female Lister Hooded rats that were chronically treated with L-DOPA post lesion. AIM=Abnormal involuntary movements, ns=not significant, SNc= Substantia nigra pars compacta, VTA=Ventral tegmental area. * $p<0.05$, ** $p<0.01$.

| | VTA | Rotations | Cylinder | AIMs |
|-----------|--------|-----------|----------|-------|
| SNc | 0.59** | n.s. | 0.34* | n.s. |
| VTA | | n.s. | n.s. | n.s. |
| Rotations | | | n.s. | 0.29* |
| Cylinder | | | | n.s. |

3.3.4. Discussion

Correlations from the male cohort

The data in Experiment 2 were used to determine whether non-pharmacological tests could be used instead of amphetamine-induced rotations to reliably balance experimental groups post-lesion, and what cut-off thresholds that could be applied to identify rats with SNc dopamine depletion $\geq 90\%$.

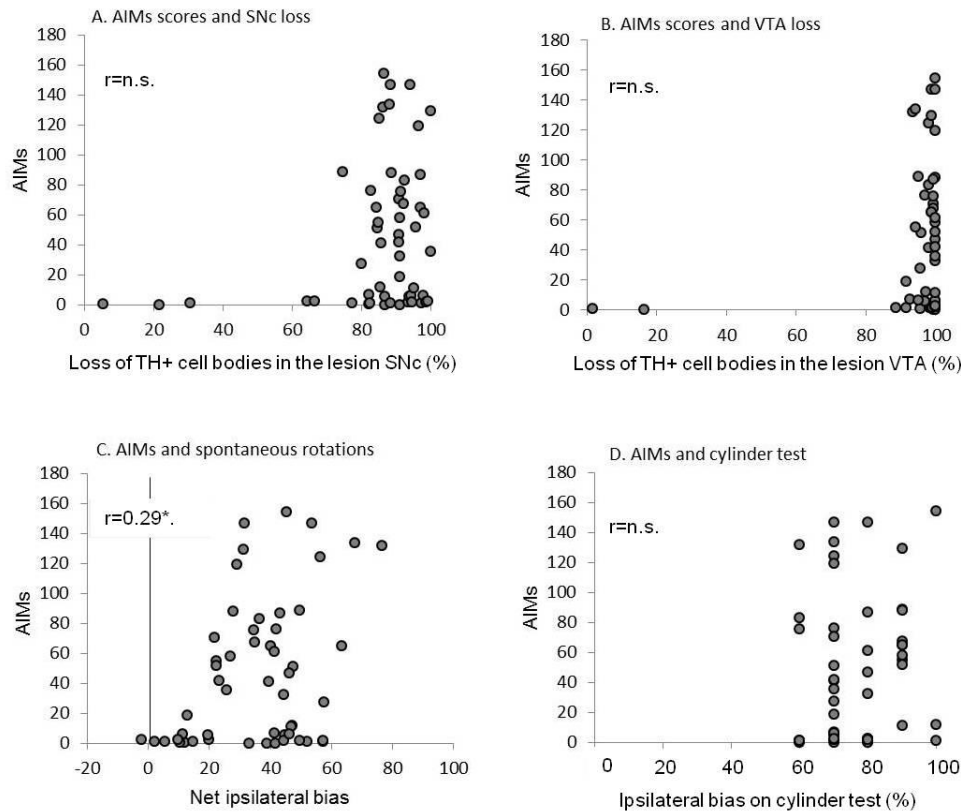


Figure 3.14. Scatterplots showing the relationship between AIMs scores and percentage loss of dopaminergic cell bodies in the ipsilateral SNc (A) and VTA (B), the net bias of spontaneous rotations (C), and ipsilateral bias on the cylinder test (D). The coefficients and the p-values of the correlations are shown in the scatterplots. AIMs=Abnormal involuntary movements; SNc=Substantia nigra pars compacta; VTA=Ventral tegmental area. * $p<0.05$.

An initial analysis including male Lister Hooded rats showed a significant correlation between dopamine loss in the SNc and ipsilateral bias on the cylinder test, spontaneous rotations, and amphetamine-induced rotations. In addition, there was a significant difference between the ipsilateral bias exhibited by rats with or without a unilateral SNc dopamine depletion of $\geq 90\%$ on the spontaneous rotations and the cylinder tests. Inspection of the data suggested that a cut-off threshold of a net ipsilateral bias of 10.0 over a 10 min period on the spontaneous rotations tests, and a 100% ipsilateral bias on the cylinder test was able to identify the vast majority of rats with SNc dopamine depletion $\geq 90\%$ whilst only falsely identifying a minority of rats showing a SNc dopamine depletion $< 90\%$.

The observed correlation between SNc dopamine loss and the ipsilateral bias on amphetamine-induced rotations ($r=0.52$) was similar to previous publications where a correlation coefficient of e.g. 0.51 ($p<0.05$) has been reported (Hudson *et al*, 1993). Interestingly, in the current experiment the correlations between SNc dopamine loss and ipsilateral bias on the cylinder ($r=0.70$) and spontaneous rotations ($r=0.64$) tests were higher than the correlation with amphetamine-induced rotations ($r=0.52$). This suggested that the cylinder and spontaneous rotation tests may be able to replace amphetamine-induced rotations as a means for balancing experimental groups post-lesion. This hypothesis was supported by the finding that there was a statistically significant difference in the performance on these tests by rats with a SNc dopamine loss of $\geq 90\%$ and $<90\%$.

The high correlation between non-pharmacological tests and lesion extent is consistent with previously published data. For example, a correlation coefficient of 0.91 ($p<0.001$) has previously been reported between ipsilateral bias in the cylinder test and dopamine loss (Schallert *et al*, 2000). Similarly, since spontaneous rotations in unilateral 6-OHDA lesion rats were first described by Ungerstedt (1968) later studies have demonstrated that a net quarter ipsilateral turns of ≥ 50 , recorded over a 15 min period in a square environment, indicate a striatal dopamine loss $\geq 85\%$ (Fornaguera *et al*, 1994), and that there is a greater rotational ipsilateral bias in 6-OHDA lesion rats with high versus low ($97.8\% \pm 0.6\%$ versus $59.6\% \pm 5.8\%$) striatal dopamine loss when recording their behaviour over night (Matsuda *et al*, 1995). Based on previous literature, it was therefore expected that the net bias of spontaneous rotations would correlate with dopamine depletion in the current experiment. However, using a partial correlation it was found that the correlation between the spontaneous net bias and SNc dopamine loss lost significance when controlling for VTA denervation, suggesting that the bias was not purely SNc driven. An involvement of the VTA in spontaneous rotations is in line with data showing that VTA denervation contributes to amphetamine- and L-DOPA-induced rotations in the nigral 6-OHDA rat lesion model, thus supporting the notion that rotational bias may not be purely SNc driven (Thomas *et al*, 1994). Despite not demonstrated to be a pure measurement of SNc dopamine loss, the spontaneous rotations test is likely to still be of value as a test used to balance experimental groups in operant studies. The VTA plays a role in non-motor functions, such as reward seeking behaviour (e.g. Fields *et al*, 2007), and being able to balance experimental groups on both SNc and VTA

denervation before commencing operant testing may therefore be highly advantageous.

The data obtained from the male Lister Hooded cohort thus suggested that (i) applying cut-off thresholds to the ipsilateral bias recorded from the spontaneous rotations and cylinder tests could identify the majority of rats with a SNc dopamine depletion $\geq 90\%$, and (ii) that ipsilateral bias on the spontaneous rotation test reflected both SNc and VTA dopamine loss. Based on these data, the cylinder and spontaneous rotation tests were used to balance experimental groups in Chapters 6-8. However, lesion rats were only excluded from the final analysis based on histological data. Thus, motor and histological data was also available from rats that did not meet the proposed cut off threshold. The data from all the rats included and excluded from the final analysis in Chapters 6-8 were therefore used to validate the findings from the male Lister Hooded cohort.

Correlations from the female cohort

A key difference between the histological data from the male and female cohort was the variation in the dopamine loss, with a lower proportion of the female rats showing a SNc dopamine loss $< 90\%$. The difference in lesion success is likely due to the lesion protocol used, which was developed using rats weighing 175-250 g (Torres *et al*, 2010). As such, the lesion coordinates used in this thesis project were likely better suited for adult female Lister Hooded rats that weigh approximately 225-310 g than for adult male Lister Hooded rats that weigh approximately 350-500 g (Charles River, 2013).

When analysing the data from the female Lister Hooded rats, only ipsilateral bias on the cylinder test correlated significantly with SNc dopamine loss. Furthermore, the cut-off thresholds that had been suggested based on data from the male cohort could no longer identify the majority of rats with SNc dopamine depletion $\geq 90\%$ without also falsely identifying a large number of rats with $< 90\%$ SNc dopamine loss. This is likely due to the more consistent lesion extent in the female rats, which is believed to have created a ceiling effect. The use of female rats may also have affected the degree of ipsilateral bias exhibited on the spontaneous rotations test as previous publications have demonstrated significantly higher amphetamine induced locomotion (Stöhr *et al*, 1998; Schindler *et al*, 2002), as well as a trend towards higher spontaneous locomotion ($p=0.06$; Stöhr *et al*, 1998), in female relative to male rats.

Data obtained from a subsample of the female rats that had received chronic L-DOPA post lesion were used to test whether the ipsilateral bias on behavioural tests correlated with, or could predict, the magnitude of future AIMs. The absence of a behavioural test that can predict susceptibility to, or future severity of, LID presents a problem to preclinical dyskinesia research. Availability of such a test would allow better balancing of experimental groups and therefore be highly valuable in e.g. research on anti-dyskinetic compounds. Therefore, an analysis exploring the correlation between the ipsilateral bias shown on the cylinder and spontaneous rotation tests and later AIMs magnitude was conducted. No significant correlation was found with between AIMs and ipsilateral bias on the cylinder test, even though such a correlation has previously been reported by Lundblad and colleagues (2002). Whereas the reason for the disparity in results has not been explicitly tested, it could potentially reflect a Type I error in the study by Lundblad and colleagues (2002) as a significant correlations between ipsilateral bias on the cylinder test and AIMs scores has, to my knowledge, not been reported by other groups.

The current data did, however, show a small but significant correlation between AIMs scores and the net bias of spontaneous rotations ($r=0.29$, $p<0.05$). While the small magnitude of the correlation suggests the test is not a good predictor of future AIMs it may still be informative for our understanding of AIMs. The partial correlation analysis in the male correlation study suggested that the net bias of spontaneous rotations was reflective of both SNc and VTA denervation. This is in line with Kelly & Moore's (1976) findings that apomorphine induced rotations are more pronounced in rats with lesions to both the striatum and NAcc, which is innervated by the VTA, than in rats with only intra-striatal lesions. In addition to innervating the NAcc, the VTA also projects to the striatum (Ferreira *et al*, 2008) and antagonizing NMDA receptors in the VTA has been shown to decrease striatal dopamine release (Karreman *et al*, 1996). AIMs, in turn, are known to increase in response to elevated extracellular striatal dopamine (Carta *et al*, 2006). An indirect effect of VTA denervation on striatal dopamine levels may thus explain why performance on a behavioural test sensitive to VTA denervation also showed a low correlation with AIMs magnitude. The small magnitude of the correlation does, however, suggest that it is an indirect relationship.

3.3.4.1. Chapter conclusions

Experiment 1 did not demonstrate any gross strain differences in the motor and operant response to L-DOPA treatment. Furthermore, although the AIMs exhibited by Lister Hooded rats were of higher magnitude than those exhibited by Sprague Dawley rats, lesion rats in both strains developed LID following chronic L-DOPA treatment. Because later experiments were interested in exploring the effect of the *onset* of LID on non-motor behaviour, rather than studying the magnitude or development of AIMs scores, it was considered valid to use Lister Hooded rats in later experiments. Importantly, the data also demonstrated that it was possible to test lesion rats that had developed LID in operant chambers following administration of 1 mg/kg acute L-DOPA without the presence of interfering AIMs. The finding informed methodological decisions in later studies where rats were administered acute L-DOPA before testing in nine-hole operant boxes (Chapters 7).

Initial findings from Experiment 2 demonstrated that non-pharmacological tests could be used in place of the conventional amphetamine-induced rotations to assess lesion success. These non-pharmacological behavioural tests hold the advantage over amphetamine-induced rotations of not potentially confounding later operant data, and were therefore used to balance experimental groups in later experiments utilizing female Lister Hooded rats. Based on retrospective data from these later experiments, a second analysis was conducted to validate the initial findings. While the analysis showed that the previously identified cut-off thresholds could not be used to successfully predict lesion success in the data from the female Lister Hooded cohort, this was potentially due to the low variation in the female cohort's dopamine denervation.

4. The Effect of Food Restriction & Chronic L-DOPA on the Motor Response to Acute L-DOPA Administration

Background: L-DOPA competes with dietary amino acids for transport across the blood-brain barrier (BBB). Therefore it was hypothesised that food restriction would increase the amount of L-DOPA that crossed the BBB and ultimately also increase the motor response to acute L-DOPA. Furthermore, chronic L-DOPA treatment sensitises striatal dopamine receptors. Therefore, chronic L-DOPA treatment was hypothesised to increase lesion rats' motor response to acute L-DOPA. To guide methodological decisions for later thesis experiments, these hypotheses were tested in two separate experiments.

Aim: Two experiments tested the hypotheses that food restriction and chronic L-DOPA treatment increase the motor response to acute L-DOPA in lesion rats.

Methods: The hypotheses were tested in both the intra-striatal and MFB 6-OHDA lesion models. Two dose response curves, conducted before and after chronic L-DOPA treatment, tested the motor response to acute L-DOPA in *ad libitum* and food restricted lesion rats using rotometers, the adjusting step test, and the vibrissae test.

Results: Food restriction was found to increase the motor response to acute L-DOPA in rats with intra-striatal but not MFB lesions. Conversely, chronic L-DOPA treatment increased the motor response to acute L-DOPA in the MFB but not the intra-striatal lesion model. Interestingly, data from the intra-striatal, but not MFB model, suggested that previous history of food restriction may increase the magnitude of subsequent AIMs. The differences between the two models are discussed in light of the differences in the extent of dopamine loss they induce and the neural regions they affect.

4.1. Introduction

L-DOPA alleviates motor symptoms arising from decreased dopaminergic innervation of the striatum. Being hydrophilic, L-DOPA can only cross the BBB by active transport across cell membranes via the L-type amino acid transporter LAT1 (del Amo *et al*, 2008; Kageyama *et al*, 2000). However, LAT1 is also implicated in the transport of dietary amino acids and the presence of the essential amino acids isoleucine, leucine, methionine, phenylalanine, tryptophan, as well as non-essential amino acids including tyrosine, significantly decreases the amount of L-DOPA accessing LAT1 transport (Kageyama *et al*, 2000).

The L-DOPA that successfully crosses the BBB is taken up by primarily striatal dopaminergic, but also some serotonergic, terminals that also contain the enzyme DOPA decarboxylase which facilitates the conversion of L-DOPA into dopamine (Flatmark *et al*, 2000). The newly synthesised dopamine is then released by dopaminergic and serotonergic terminals into extracellular space (Tanaka *et al*, 1999; Cenci & Lundblad, 2006; Lindgren *et al*, 2010). The subsequent effect of L-DOPA on motor function is dependent on both the amount of dopamine available and the sensitivity of the postsynaptic dopamine receptors.

When evaluating motor and non-motor function in rat models of PD certain behavioural tasks, such as the LCRT paradigm used in Chapters 7 and 8 (section 2.7.2.2), require that food intake is restricted during operant training and testing. Considering the competition between L-DOPA and dietary amino acids for LAT1 transport, restricted food intake is likely to decrease L-DOPA's competition for transport across the BBB. This, in turn, may increase the overall amount of L-DOPA that enters the brain and, ultimately, the amount of dopamine that can be synthesised. It was therefore hypothesised that food restricted lesion rats would show an increased motor response to acute L-DOPA relative to *ad libitum* controls. If true, dose response curves based on data from *ad libitum* rats may not be appropriate when selecting a therapeutic dose of L-DOPA to be administered to food restricted rats.

Following extensive depletion of nigrostriatal dopamine, as induced in the rat 6-OHDA model, there is a change in receptor populations, resulting in decreased binding to D1 type receptors and increased binding to D2 type receptors (Xu *et al*. 2005). However, chronic administration of L-DOPA to lesion rats sensitises D1 type

receptors and also increases the rotational response to acute L-DOPA administration in rats that develop LID (Konradi *et al*, 2004; Henry *et al*, 1998).

Long-term L-DOPA treatment also causes sensitization of D1 receptors and an increased contralateral rotational bias in response to later administration of acute L-DOPA (Henry *et al*, 1998; Cenci *et al* 2006; Konradi *et al*, 2004; Aubert *et al*, 2005). Hence, rats that are L-DOPA naive or have been chronically treated with L-DOPA differ both in terms of the sensitivity of their dopamine receptors and their behavioural response to L-DOPA. Based on this, it was hypothesised that a lower L-DOPA dose would be required to produce a motor response in rats that had been chronically treated with L-DOPA relative to rats with little or no previous L-DOPA exposure. Therefore, both experiments described in this chapter included two dose response curves; generated before and after chronic L-DOPA treatment respectively. Chronic L-DOPA treatment was defined as the duration of treatment required for the groups to show stable AIMs scores.

It should be noted that Experiment 3 commenced prior to Experiments 1 and 2 (Chapter 3) and was therefore designed in the absence of data showing whether Lister Hooded rats were suitable for L-DOPA studies; or whether non-pharmacological lesion screening tests could reliably be used instead of amphetamine-induced rotations. To ensure consistency with previous in-house L-DOPA experiments that had been conducted using the same apparatus (e.g. Monville *et al*, 2005), the experiments in this chapter therefore used Sprague Dawley rats and lesion success was screened using amphetamine-induced rotations.

Furthermore, the chapter reports both data from rats with intra-striatal (Experiment 3) and MFB (Experiment 4) lesions. Both models have been successfully utilised in the LCRT task that was planned to be used in later thesis chapters (e.g. Dowd & Dunnett, 2004, 2005a,b) and were therefore candidate lesion models for later thesis project experiments. The site of 6-OHDA infusion affects both the degree of dopamine denervation that is observed and the extent to which adjacent regions are affected. The intra-striatal 6-OHDA model causes focal loss of dopamine around the part of the striatum into which the infusion is administered. Conversely, infusing 6-OHDA into the MFB induces almost complete unilateral denervation of ipsilateral nigral and striatal dopamine as well as reduced dopamine levels in the VTA and associated projection areas in the ventral striatum (Yuan *et al*, 2005; Deumens, 2002).

The conversion of L-DOPA to dopamine predominantly occurs in striatal dopaminergic terminals. It was therefore hypothesised that the near-complete striatal dopamine loss induced by MFB lesions would result in a ceiling effect, in which the limited number of remaining dopaminergic terminals in the lesion striatum would reduce the amount of L-DOPA that was able to be converted into dopamine. Ultimately, regardless of whether or not food restricted rats had a greater L-DOPA influx than *ad libitum* controls, this may be hypothesised to reduce the chance of observing a difference in food restricted and *ad libitum* MFB lesion rats' motor response to acute L-DOPA. Conversely, the relative sparing of striatal dopaminergic terminals in the intra-striatal, relative to the MFB, model was expected to decrease the risk of a ceiling effect. Hence, it was believed that the greater preservation of dopaminergic terminals observed in the intra-striatal model would allow greater uptake and conversion of striatal L-DOPA to dopamine, and therefore increase the likelihood of observing a difference between the food restricted and *ad libitum* cohort. Testing the hypothesis in both lesion models thus provided a more thorough testing of the hypothesis that food restriction increases L-DOPA influx in the rat.

4.2 Experiment 3: The effect of food restriction and chronic L-DOPA on the motor response to acute L-DOPA in rats with intra-striatal 6-OHDA lesions

4.2.1. Introduction

An initial dose-response curve was conducted in rats with intra-striatal lesions. In line with the chapter hypotheses, two dose response curves were conducted to compare the motor response of *ad libitum* and food restricted lesion rats, before and after chronic L-DOPA treatment. It was hypothesised that both food restriction and chronic L-DOPA would increase the motor response to the drug.

4.2.2. Aims

Two dose response curves were generated to measure the motor response to acute L-DOPA following (i) food restriction, and (ii) chronic L-DOPA treatment.

4.2.2. Methods

4.2.2.1. Experimental Design

Rats were given unilateral 6-OHDA infusions into the striatum. Lesion extent was assessed using amphetamine-induced rotations. Based on the net rotational bias, the cohort was divided into an *ad libitum* (n=7) and a food restricted (n=7) group and their motor response to saline and seven L-DOPA doses was assessed using automated rotometers, the adjusting step test and the vibrissae test. Following an initial dose response curve, rats were chronically treated with L-DOPA while allowed *ad libitum* access to food, and their AIMs were recorded. Following chronic L-DOPA treatment, the previously food restricted (n=7) group was once again food restricted and they and the *ad libitum* (n=7) group were used to generate a second dose response (Figure 4.1).

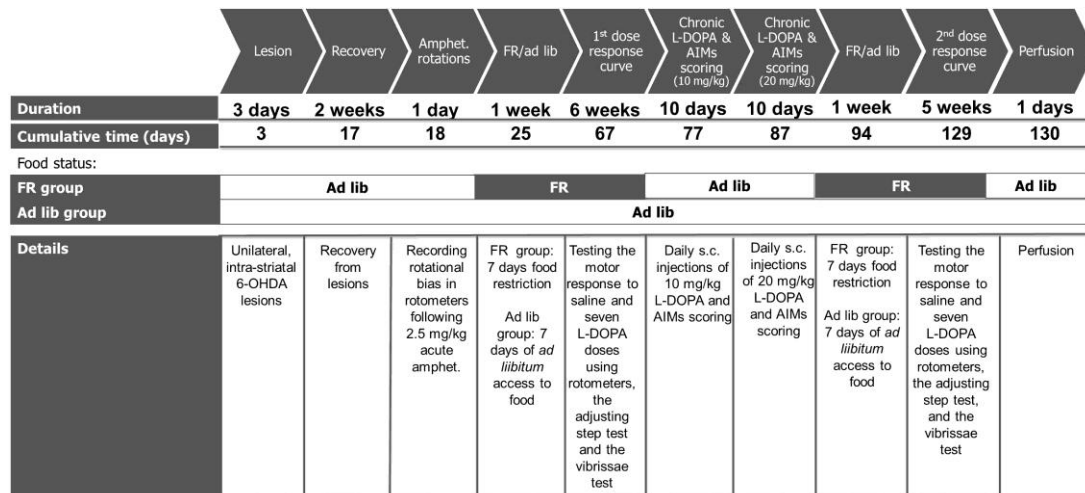


Figure 4.1. A graphical representation of the experimental design of Experiment 3. The figure specifies both the duration of the individual testing phases and the overall number of days in the experiment. The 'food status' boxes indicate when in the experiment the respective groups were given restricted (grey boxes) or *ad libitum* (white boxes) access to food. Ad lib=*ad libitum*; AIMs=Abnormal involuntary movements; Amphet.=amphetamine; FR=food restriction; s.c.=subcutaneous.

4.2.2.2. Procedure

Female Sprague Dawley rats (n=14) were given unilateral lesions to the striatum (section 2.2.3). Following a two week recovery period, amphetamine-induced rotations (section 2.4.6.2) were conducted to assess lesion extent and the results used to balance the groups. As previously stated, amphetamine-induced rotations were used

because this experiment was conducted prior to Experiment 2 where it was demonstrated that ipsilateral bias on the cylinder test and spontaneous rotations showed a stronger correlation with dopamine loss in a unilateral 6-OHDA model than amphetamine-induced rotations did.

Two dose response curves were generated, using saline and seven L-DOPA doses (0.5, 1.0, 2.5, 5.0, 6.25, 7.5 and 10.0 mg/kg) administered in a counter-balanced order. The first dose response curve was generated using L-DOPA naïve rats. The second dose response curve was generated using the same rats after these had received chronic L-DOPA treatment. During the chronic L-DOPA period, rats received daily s.c. injections of 10 mg/kg L-DOPA for a period of 10 days. This was followed by 10 days of daily s.c. injections with 20 mg/kg L-DOPA, to ensure that rats exhibited the maximum possible AIMs scores. Throughout the chronic treatment period, rats were scored for AIMs as described in section 2.5.

Motor behaviour data were generated using automated rotometers (section 2.4.6.1), the adjusting step test (section 2.4.3), and the vibrissae test (section 2.4.8). At the start of testing, rats were placed in automated rotometers that were pre-set to record rotational behaviour for 90 min and turned on immediately following a s.c. injection of saline or L-DOPA. Twenty min post-injection, rats were temporarily removed from the rotometers and tested on the adjusting step test and the vibrissae test in an adjacent room. Immediately after hand testing, rats were returned to the rotometers until all rotational data had been collected. The adjusting step and vibrissae tests were selected because they have previously been shown sensitive to the therapeutic effect of dopaminergic drugs on motor behaviour (Winkler *et al*, 2002; Pinna *et al*, 2010). Furthermore, because of the non-spontaneous nature of the adjusting step and vibrissae tests, utilization of these tests made it possible to obtain a motor response from all rats in a minimum period of time, thereby increasing the time available to record L-DOPA induced rotations in the rotometers, something that could not have been guaranteed had e.g. the cylinder test been used. A wash-out period of a minimum of two days was allowed between each testing day.

Following completion of behavioural testing, rats were given an overdose of Euthatal, perfused (section 2.8), and the brain tissue taken for TH immunohistological staining (section 2.10). The lesion extent was determined by counting TH positive cells in the SNc, as described in section 2.11, and by measuring TH optical density of the ipsilateral and contralateral striatum using Image J (section 2.12).

4.2.2.3. Statistical analysis

Striatal optical density was analysed using two separate tests. First, the raw optical density measures from the ipsilateral versus contralateral striatum were compared using a t-test to confirm that the lesion had reduced TH+ staining in the lesion striata. Second, a one-way ANOVA with Group as between-subject factor was used to test whether the experimental groups differed in the percentage loss of ipsilateral striatal TH+ immunoreactivity induced by the lesion.

The effect of the lesion on the number of TH+ cell bodies in the SNc and VTA was also analysed using two separate types of analyses. First, the presence of a lesion in the SNc or VTA was confirmed by comparing the raw cell counts in the ipsilateral and contralateral hemisphere using a t-test. For this analysis, the SNc and VTA were analysed separately. Once the presence of a lesion had been confirmed, an ANOVA was used to test whether there was a difference in (i) the relative extent of the experimental groups' dopamine denervation, and (ii) the loss of dopaminergic cell bodies observed in the SNc versus the VTA. The ANOVA was based on the percentage loss of TH+ cell bodies observed in the lesion SNc and VTA and included Region as a within-subject variable, and Group as a between-subject variable.

To analyse the effect of Food restriction, motor data from the dose response curves were analysed using a repeated measures ANOVA. Data from the dose response curves generated before and after chronic L-DOPA treatment were analysed separately. For the adjusting step and vibrissae tests, data from the ipsilateral and contralateral performance were included in the same analysis. Significant between-subject effects were analysed using a Tukey post-hoc test and significant interactions were analysed using a test of simple effects.

To analyse the effect of chronic L-DOPA treatment on the motor response to subsequent acute L-DOPA administration, the motor data collected before and after chronic L-DOPA treatment were compared using a repeated measures ANOVA. When analysing the rotational bias data, Chronic L-DOPA and Dose were used as within-subject factors. When analysing the adjusting step and vibrissae test data, Side, Chronic L-DOPA, and Dose were used as within-subject factors.

AIMs scores were analysed using a repeated measures ANOVA with Group as a between-subject factor and Dose as a within-subject factor.

4.2.3. Results

4.2.3.1. Dopamine depletion

The success of the intra-striatal lesion was demonstrated by a decrease in the optical density in the lesion, relative to the intact, striatum (Figure 4.2A) as well as a loss in dopaminergic cell bodies in the SNc and VTA (Figure 4.2B). The median optical density in the lesion striatum was 53% (sem: 9%) of that measured in the intact striatum and the number of dopaminergic cell bodies remaining in the lesion SNc and VTA were 24% (sem: 4%) and 61% (sem: 7%) of that in the intact side.

Comparison between the optical density measured in the intact and lesion striatum confirmed that the 6-OHDA lesion had induced a significant loss of striatal immuno-reactivity in the ipsilateral hemisphere ($t_{13}=5.14$, $p<0.001$). A further analysis was conducted to test whether there was a difference in the percentage reduction in striatal immunoreactivity observed in the *ad libitum* and food restricted groups. These data confirmed that the percentage reduction in ipsilateral striatal immune-reactivity was similar between the two groups ($F_{1,12}=0.09$, n.s.).

Subsequent analyses tested the effect of the lesion on loss of dopaminergic cell bodies in the SNc and VTA. Analysis of the raw cell counts demonstrated that both groups had significantly fewer dopaminergic cell bodies in the lesion than the intact SNc ($t_{13}=9.55$, $p<0.001$) and VTA ($t_{13}=4.01$, $p<0.01$). A further analysis was conducted to test whether there was a difference in the percentage loss of dopaminergic cell bodies observed in the lesion SN and VTA, and whether the groups' differed in their dopamine denervation in these structures. The data confirmed that the lesion induced a significantly greater percentage loss of dopaminergic cell bodies in the SNc than the VTA (Region: $F_{1,12}=16.74$, $p<0.001$), and that the percentage loss of dopaminergic cell bodies was similar in the *ad libitum* and food restricted groups (Region x Group: $F_{1,12}=0.91$, n.s.; Group: $F_{1,12}=0.10$, n.s.). Based on the data in this and the preceding paragraph, the lesion was considered to have been successful and the groups to be appropriately matched.

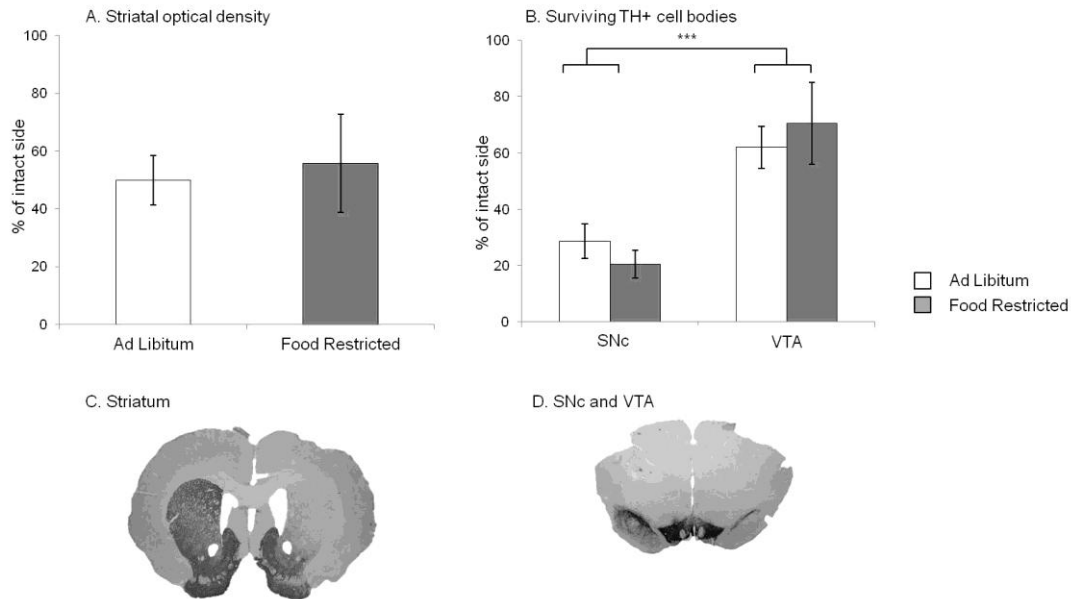


Figure 4.2. Striatal optical density (A) and dopaminergic cell bodies in the SNc and VTA (B), both expressed as a percentage of the intact side, of the *ad libitum* (n=7) and food restricted (n=7) group, together with photos showing a striatum (C) and SNc and VTA (D) from a lesion rat included in the experiment. The bars show the mean, and the error bars the standard error of the mean. SNc=Substantia nigra pars compacta, TH=Tyrosine hydroxylase, VTA=Ventral tegmental area. ** $p < 0.01$

4.2.3.2. First dose response curve

Net rotations. An initial response curve was conducted to show the motor response to acute L-DOPA in rats with little or no L-DOPA exposure. Despite a trend towards an overall greater contralateral rotational bias in food restricted than *ad libitum* rats (Figure 4.3A; Group: $F_{1,12}=4.22$, $p=0.062$), the difference only reached statistical significance following administration of specific L-DOPA doses. Specifically, the food restricted rats showed a greater contralateral net bias than the *ad libitum* controls following administration of 6.25 mg/kg L-DOPA (Dose: $F_{7,84}=4.71$, $p < 0.001$; Dose x Group: $F_{7,84}=3.83$, $p < 0.01$; Simple Effect of Group on Dose: $F_{1,12}=5.63$, $p < 0.005$). In addition, there was a trend towards a difference in the net rotational bias of the food restricted relative to the *ad libitum* control group following acute administration of saline and 1.0 mg/kg L-DOPA. At these doses the *ad libitum* rats showed a net ipsilateral rotational bias, whereas the food restricted rats did not demonstrate any rotational bias (Simple Effect of Group on Dose: min. $F_{1,12}= 4.31$, $p=0.06$ and 0.054 respectively). There was also a trend towards a greater contralateral bias in the food

restricted relative to the *ad libitum* control group following acute administration of 7.5 mg/kg L-DOPA (Simple Effect of Group on Dose: $F_{1,12}=4.54$ $p=0.055$).

Adjusting step test. Both groups exhibited a contralateral deficit in the adjusting step test (Figure 4.3C,E; Side: $F_{1,12}=107.07$, $p<0.01$). While there was a significant overall effect of L-DOPA on adjusting step test performance, visual inspection of the data suggested this was due to random fluctuations in the data which did not relate to the experimental hypothesis (Figure 4.3E; Dose: $F_{7,84}=2.73$, $p<0.05$; Group: $F_{1,12}=1.88$, n.s.; Side x Group: $F_{1,12}=3.38$, n.s.; Dose x Group: $F_{7,84}=0.73$, n.s.).

Vibrissae test. Both groups showed an impaired contralateral vibrissae reflex (Figure 4.3G, I; Side: $F_{1,12}=19.16$, $p<0.01$; Side x Group: $F_{1,12}=2.86$, n.s.). Acute L-DOPA improved both groups' contralateral performance, without affecting ipsilateral responses (Side x Dose: $F_{7,84}=2.73$, $p<0.05$; Simple effect of Dose on Side: $F_{7,6}=7.68$, $p<0.05$; Dose x Group: $F_{7,84}=1.17$, n.s.). While there was a trend towards a higher performance in the food restricted relative to the *ad libitum* control group it did not meet statistical significance (Group: $F_{1,12}=4.33$, $p=0.06$; Side x Group: $F_{1,12}=2.86$, n.s.).

4.2.3.2. Second dose response curve

Net Rotations. A second dose response curve was conducted at the end of the chronic L-DOPA treatment. The food restricted group showed an overall greater rotational response to acute L-DOPA than the *ad libitum* control group, in which no rotational bias was observed (Figure 4.3B; Group: $F_{1,12}=6.90$, $p<0.05$). The effect of food restriction was significant following administration of L-DOPA doses 7.5 and 10.0 mg/kg (Dose: $F_{7,84}=5.59$, $p<0.001$; Dose x Group: $F_{7,84}=5.22$, $p<0.001$; Simple Effect of Group on Dose: $\min.F_{1,12}=7.22$, $p<0.05$).

Adjusting step test. The rats continued showing a significant, albeit modest, contralateral deficit in the adjusting step test during the second dose response curve (Figure 4.3D,F; Side: $F_{1,12}=23.44$, $p<0.001$; Dose: $F_{7,84}=0.77$, n.s.; Dose x Group: $F_{7,84}=1.47$, n.s.; Side x Dose: $F_{7,84}=1.77$, n.s.). The *ad libitum* group showed a subtle, but significantly higher performance than the food restricted group when tested on their ipsilateral, but not their contralateral, side (Group: $F_{1,12}=4.94$, $p<0.05$; Side x Group: $F_{1,12}=10.27$, $p<0.01$; Simple effect Group on Ipsilateral side: $F_{1,12}=8.13$, $p<0.05$).

The Effect of Food Restriction on the Motor Response to Acute L-DOPA

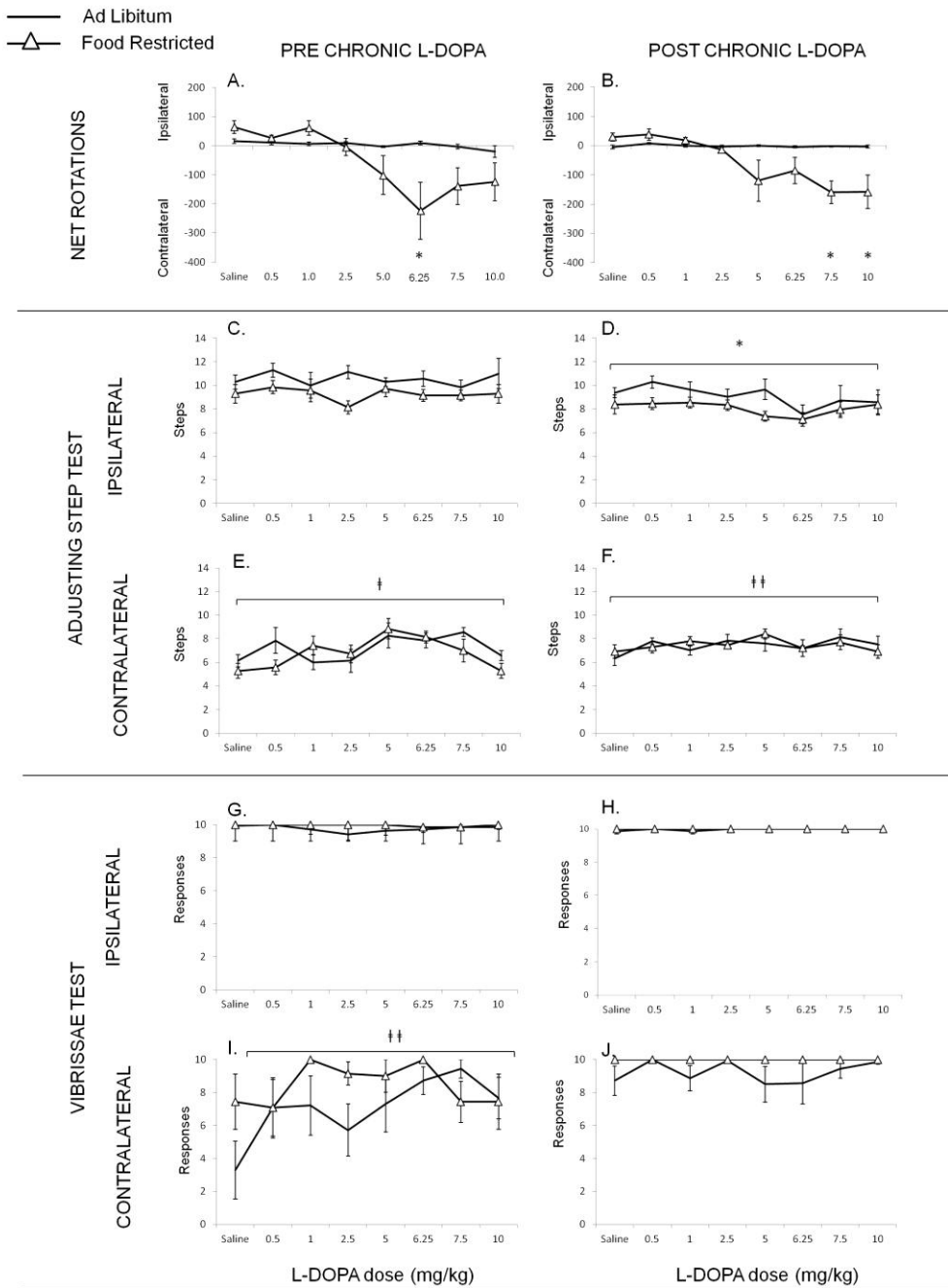


Figure 4.3. The motor effect of acute L-DOPA on net rotational bias (A-B), the adjusting step test (C-F), and the vibrissae (G-J) test in rats with intra-striatal lesions that had *ad libitum* (n=7) or restricted (n=7) access to food. The dose response curves were generated before (left panel) and after (right panel) chronic L-DOPA treatment. The line charts show the mean and the error bars show the standard error of the mean. *Effect of Food restriction, †Effect of Lesion, *p<0.05, ‡p<0.05, ††p<0.01

Vibrissae test. Following chronic L-DOPA treatment, the previously observed contralateral deficit in the vibrissae test disappeared (Figure 4.3H,J; Side: $F_{1,12}=4.25$, n.s.; Side x Group: $F_{1,12}=4.25$, n.s.). Whilst there was a trend towards a overall higher performance in the *ad libitum* relative to the food restricted group, this did not meet statistical significance (Group: $F_{1,12}=4.41$, $p=0.058$) and because visual inspection of the data suggested that the trend did not reflect dose dependent change in performance, it was not considered relevant for the hypothesis being tested. Acute L-DOPA administration did not affect performance (Dose: $F_{7,84}=0.73$, n.s.; Dose x Group: $F_{7,84}=0.73$, n.s.; Side x Dose: $F_{7,84}=0.94$, n.s.). This was likely due to a ceiling effect, as neither group exhibited a contralateral deficit after chronic L-DOPA treatment.

4.2.3.3. Abnormal involuntary movements

Between the first and second dose response curve, all rats were chronically treated with 10 mg/kg L-DOPA until showing stable AIMs. Interestingly, despite all rats being given *ad libitum* access to food during chronic L-DOPA treatment, the previously food restricted group exhibited higher AIMs scores than the *ad libitum* control group (Figure 4.4A). To test whether both groups were exhibiting their maximum achievable AIMs, or if the group difference would disappear if AIMs could be increased, the daily dose of L-DOPA injection was increased to 20 mg/kg L-DOPA. After 10 days of daily administration of 20 mg/kg L-DOPA, a final AIMs scoring session was conducted. Rats showed AIMs of similar magnitude as when they had been administered 10 mg/kg, suggesting that these were indeed the maximum AIMs that could be induced in them and that previously food restricted rats did indeed have higher AIMs than *ad libitum* controls (Group: $F_{1,12}=12.38$, $p<0.01$; Dose: $F_{1,12}=0.02$, n.s.; Dose x Group: $F_{1,12}=1.10$, n.s.).

To ensure that the data was not due to experimenter bias the AIMs were also scored by a second, blinded, scorer. The AIMs scores obtained by the blinded scorer confirmed that the previously food restricted rats showed higher AIMs than the *ad libitum* controls, and that these AIMs were similar when administered 10 mg/kg and 20 mg/kg L-DOPA (Figure 4.4B; Group: $F_{1,12}=11.40$, $p<0.01$; Dose: $F_{1,12}=4.13$, n.s.; Dose x Group: $F_{1,12}=4.68$, n.s.).

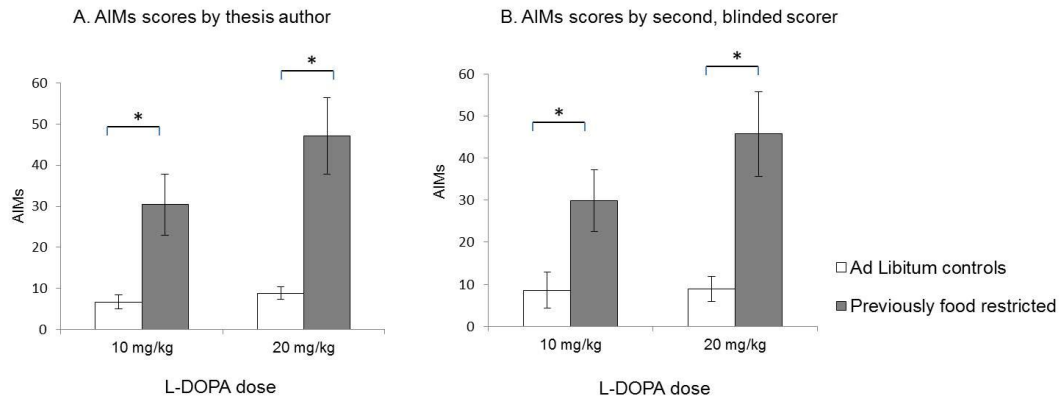


Figure 4.4. The cumulative AIMs scores of the *ad libitum* control group (n=7) and the previously food restricted group (n=7) when scored by the thesis author (A) and a blinded (B) scorer following administration of 10 mg/kg and 20 mg/kg L-DOPA. The bars show the mean and the error bars the standard error of the mean. AIMs=Abnormal involuntary movements. *p<0.05

4.2.3.4. The effect of chronic L-DOPA treatment on motor function

To test whether acute L-DOPA induced a greater motor response in rats that had little L-DOPA exposure or that had a history of chronic L-DOPA treatment, a second dose response curve was conducted at the end of the chronic L-DOPA treatment. The following section presents a comparison of the motor response observed before, and after, chronic L-DOPA when data from all rats were analysed together.

Net Rotations. Rats' net rotational response to acute L-DOPA was of similar magnitude both before and after chronic L-DOPA treatment (Figure 4.5A; Chronic L-DOPA: $F_{1,12}=0.05$, n.s.; Chronic L-DOPA x Dose: $F_{7,91}=0.95$, n.s.).

Adjusting Step Test. Chronic L-DOPA treatment did not affect the overall number of steps made on the adjusting step test (Figure 4.5B-C; Chronic L-DOPA: $F_{1,13}=4.35$, n.s.; Chronic L-DOPA x Dose: $F_{7,91}=1.68$, n.s.). While there was a decrease specifically in the number of ipsilateral steps made after, relative to before, chronic L-DOPA treatment,

The Effect of Chronic L-DOPA on the Motor Response to subsequent acute L-DOPA

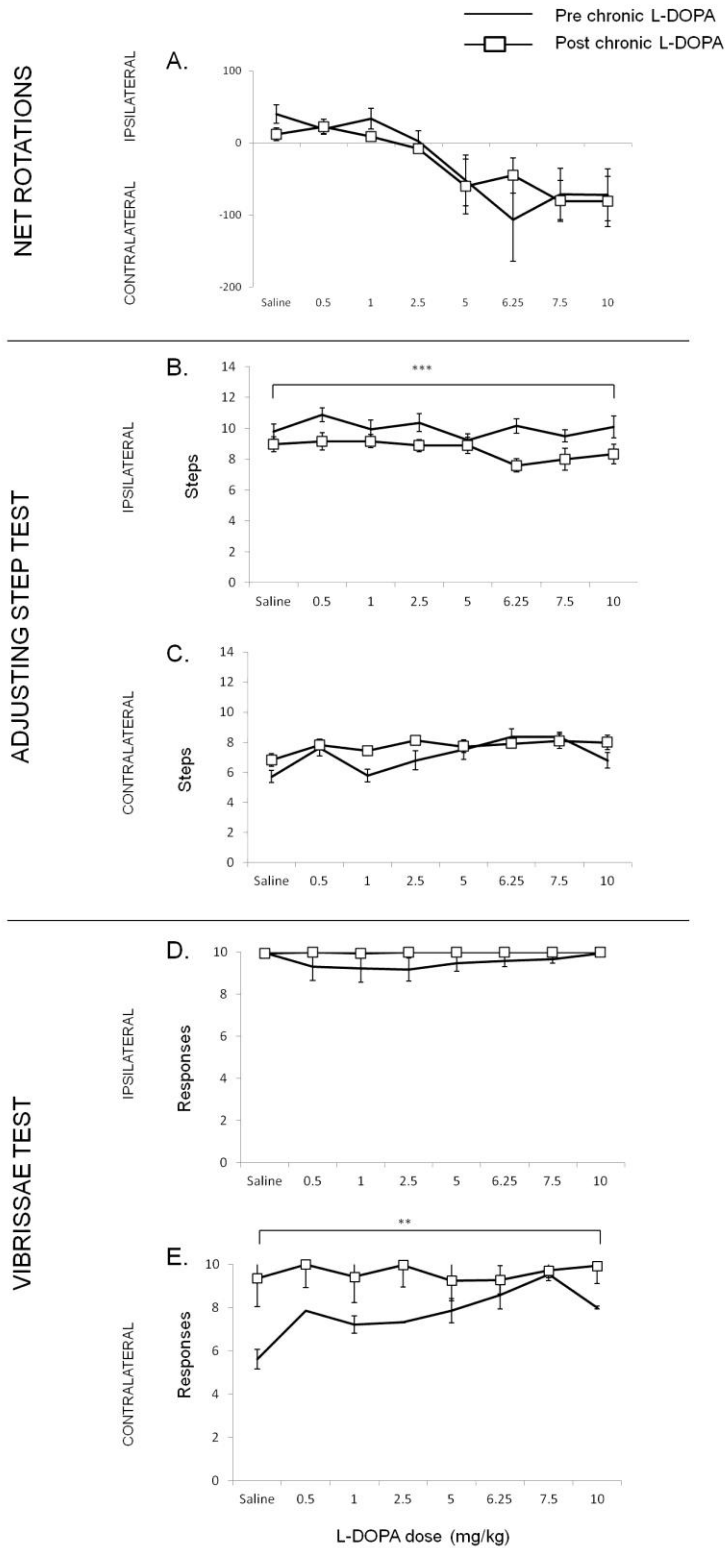


Figure 4.5. The net rotational bias (A), and performance on the adjusting step (B-C) and the vibrissae tests (D-E) before and after chronic L-DOPA treatment in rats with intra-striatal lesions (n=14). The line charts show the mean, and the error bars show the standard error of the mean. **p<0.01, ***p<0.001.

the actual difference was small and unlikely to be of relevance for the experiment hypothesis; which predicted a greater contralateral response after than prior to chronic L-DOPA (Chronic L-DOPA x Side: $F_{1,13}=19.70$, $p<0.01$; Simple Effect of Chronic L-DOPA on Side: $F_{1,13}=7.51$, $p<0.05$).

Vibrissae test. Chronic L-DOPA treatment caused an overall improvement, exclusively in contralateral performance (Figure 4.5D,E; Chronic L-DOPA: $F_{1,13}=28.71$, $p<0.001$; Chronic L-DOPA x Side: $F_{1,13}=24.22$, $p<0.001$; Simple Effect of Chronic L-DOPA on Side: $F_{1,13}=16.76$, $p<0.01$). Visual inspection of the data suggested that the statistically significant effect of chronic L-DOPA was due to the disappearance of the contralateral deficit in the second dose response curve (Side: $F_{1,13}=12.52$, $p<0.01$; Chronic L-DOPA x Dose: $F_{7,91}=1.80$, n.s).

4.3. Experiment 4: The effect of food restriction and chronic L-DOPA on the motor response to acute L-DOPA in rats with MFB 6-OHDA lesions

4.3.1. Introduction

It had been hypothesized that food restriction would increase the transport of L-DOPA across the BBB, and that this would in turn increase the motor response to acute L-DOPA. Unexpectedly, Experiment 3 also suggested an effect of a history of food restriction on the magnitude of subsequent AIMs. However, based on data from the previous experiment, it was not clear whether the effect of food restriction on later AIMs was due to a long-lasting effect of food restriction itself or if it was caused by an interaction between food restriction and L-DOPA exposure. In addition to testing the chapter hypotheses, the current experiment therefore also further explored the previously observed effect of food restriction on subsequent AIMs magnitude by comparing AIMs scores between rats that had (i) never been food restricted, (ii) been food restricted during previous L-DOPA exposure, or (iii) only been food restricted in the absence of L-DOPA.

4.3.2. Aims

The experiment tested (i) the effect of food restriction on the motor response to acute L-DOPA, (ii) the effect of chronic L-DOPA on the motor response to subsequent acute L-DOPA, and (iii) whether previous food restriction impacted on the magnitude

of AIMs observed when later administered chronic L-DOPA while allowed *ad libitum* access to food.

4.3.2. Methods

4.3.2.1. Experimental design

Twenty-four rats were given unilateral 6-OHDA lesions to the MFB. Following recovery the lesion extent was assessed using amphetamine-induced rotations. Based on their net rotations, rats were divided into five subgroups and two dose response curves were generated. Between the two dose response curves was one drug free interval period followed by chronic L-DOPA treatment. All groups were given *ad libitum* access to food during chronic L-DOPA treatment. However, the groups differed from each other in the duration and timing of their food restriction during the other testing phases, as illustrated in Figure 4.6. The ‘*ad libitum* control’ group (n=9) was never food restricted. The ‘FR control’ group (n=4) was food restricted during the first and second dose response curve, but not during the drug free interval. The ‘FR 1’ group (n=5) was food restricted during the first dose response curve but given *ad libitum* access to food through the remaining testing phases. The ‘FR interval’ group (n=3) was food restricted during the drug-free interval but given *ad libitum* access during the two dose response tests. The ‘FR interval + 2’ group (n=3) was food restricted during the drug-free interval and second dose response curve, but given *ad libitum* access to food during the first dose response curve. In the first dose response curve there were thus 15 *ad libitum* rats and 9 food restricted rats whereas the second dose response curve included 17 *ad libitum* rats and 7 food restricted rats. As previously stated, all rats were allowed *ad libitum* access to food during the chronic L-DOPA phase. The purpose of the design was to test whether there was an effect of food restriction on later AIMs as suggested in Experiment 3 while also testing whether there was a difference between the AIMs observed in rats food restriction in the absence or presence of L-DOPA.

Hence, the first dose response curve compared the motor response of rats receiving *ad libitum* (total n=15) or restricted (total n=9) food access to saline and six L-DOPA doses using automated rotometers, the adjusting step test and the vibrissae test. A second dose response curve was then conducted, comparing the motor response of rats with *ad libitum* (total n=17) or restricted (total n=7) food access to

saline and the same six L-DOPA doses using the same motor tests as the initial dose response curve.

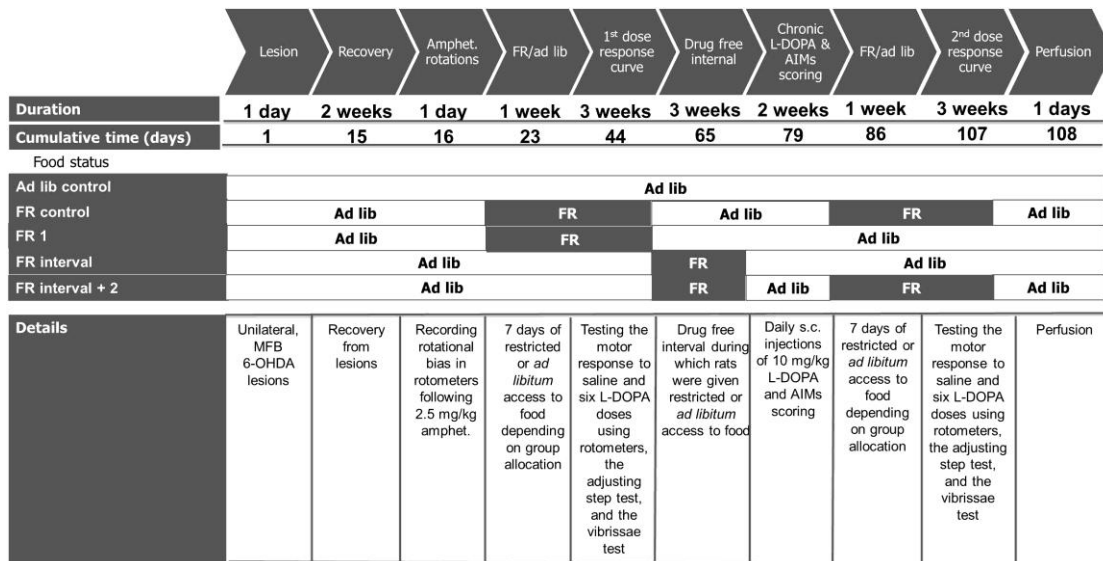


Figure 4.6. A graphical representation of the experimental design of Experiment 4. The 'food status' boxes indicate when in the experiment the respective groups were given restricted (grey boxes) or *ad libitum* (white boxes) access to food. Ad lib=*ad libitum*; AIMS=Abnormal involuntary movements; Amphet.=amphetamine, FR=food restriction; s.c.=subcutaneous.

4.3.2.2. Procedure

Female Sprague Dawley rats (n=24) were given unilateral lesions to the MFB (section 2.2.2). Following a two week recovery period, amphetamine-induced rotations (section 2.4.6.2) were conducted to assess lesion extent and the results used to balance the groups.

Two dose response curves including saline and six L-DOPA doses (0.5, 1.0, 2.5, 5.0, 7.5 and 10.0 mg/kg) were conducted using the same procedure as in Experiment 3 (section 4.2.3.2). The 6.5 mg/kg dose that had been included in the intra-striatal Experiment 3 was not included in the current dose response curves. The decision to exclude the dose was based on the fact that there was little difference between the L-DOPA concentration in this and adjacent doses. Excluding it thus increased animal welfare by reducing unnecessary drug exposure without compromising the experiment.

4.3.2.3. Statistical analysis

Striatal optical density was analysed using two separate, non-parametric tests. First, the raw optical density measures from the ipsilateral versus contralateral striatum were compared using a Wilcoxon test to confirm that the lesion had reduced TH+ staining in the lesion striata. Second, a Mann-Whitney U-test was used to test whether the experimental groups differed in the percentage loss of ipsilateral striatal TH+ immunoreactivity induced by the lesion.

The effect of the lesion on the number of TH+ cell bodies in the SNc and VTA was also analysed using two separate types of analyses. First, the presence of a lesion in the SNc or VTA was confirmed by comparing the raw cell counts in the ipsilateral and contralateral hemisphere using a t-test. For this analysis, the SNc and VTA were analysed separately. Once the presence of a lesion had been confirmed, an ANOVA was used to test whether there was a difference in (i) the relative extent of the experimental groups' dopamine denervation, and (ii) the loss of dopaminergic cell bodies observed in the SNc versus the VTA. The ANOVA the percentage loss of TH+ cell bodies observed in the lesion SNc and VTA and included Region as a within-subject variable, and Group as a between-subject variable.

To analyse the effect of Food restriction, motor data from the dose response curves were analysed using a repeated measures ANOVA. Data from the dose response curves generated before and after chronic L-DOPA treatment were analysed separately. For the adjusting step and vibrissae tests, data from the ipsilateral and contralateral performance were included in the same analysis. Significant interactions were analysed using a test of simple effects. Because of a significant difference between the *ad libitum* and food restricted groups' VTA depletion during the first dose response curve, VTA depletion was included as a covariate when analysing behavioural data from the first dose response curve.

The final AIMs scores data were analysed using a one-way ANOVA, whereas the development of AIMs was analysed using a repeated measures ANOVA with Group as a between-subject variable and Day as a within-subject factor.

To analyse the effect of chronic L-DOPA treatment on the motor response to subsequent acute L-DOPA, the motor data from before and after chronic L-DOPA treatment were compared using a repeated measures ANOVA. When analysing the rotational bias data, Chronic L-DOPA and Dose were used within-subject factors.

When analysing the adjusting step and vibrissae test data, Side, Chronic L-DOPA, and Dose were used as within-subject factors.

4.3.3. Results

4.3.3.1. Dopamine depletion

As further described in the subsequent results section, the motor and AIMs data from all rats that had *ad libitum* or restricted access to food in the test phase being analysed (i.e. the first dose response curve, the chronic L-DOPA phase, and the second dose response curve) were pooled in the statistical analysis. However, as illustrated in Figure 4.6, the time of food restriction differed between the experimental groups and, as a result, the total number *ad libitum* or food restricted rats was different in the three testing phases. Therefore, once statistical analysis had confirmed that the lesion reduced optical density and dopaminergic cell bodies in the lesion hemisphere, three further analyses were conducted to test whether there was a difference between the dopamine denervation in food restricted and *ad libitum* rats. Each analysis was restricted to one phase of experiment. For simplicity, the difference *ad libitum* and food restricted rats' striatal immunoreactivity and SNc and VTA denervation is discussed in separate paragraphs.

Overall lesion success. The lesion successfully reduced the number of dopaminergic cell bodies observed in the lesion relative to the intact SNc ($t_{23}=14.38$, $p<0.001$) and VTA ($t_{23}=17.28$, $p<0.001$), as well as the optical density measured in the ipsilateral relative to the contralateral striatum (Figure 4.7H; $Z=-4.29$, $p<0.001$)

Histological data from the first dose response curve. Overall, there was a greater percentage loss of cell bodies in the lesion SNc than in the lesion VTA (Figure 4.7A; Region: $F_{1,22}=46.53$, $p<0.001$). This VTA denervation was significantly more pronounced in the *ad libitum* than in the food restricted group (Group: $F_{1,22}=5.93$, $p<0.05$; Group x Region: $F_{1,22}=4.84$, $p<0.05$; Simple Effect of Group on VTA: $F_{1,22}=5.63$, $p<0.05$). The groups were, however, matched on the other measures of dopamine denervation, i.e. percentage loss of cell bodies in the lesion SNc (Simple Effect of Group on VTA: $F_{1,22}=1.62$, n.s.) and percentage reduction in the optical density of the lesion striata (Figure 4.7E; $U=41.00$, n.s.).

Histological data from the drug free interval & chronic L-DOPA treatment phase. The percentage loss of dopaminergic cell bodies in the SNc and VTA was similar for *ad libitum* and food restricted rats (Figure 4.7B; Group:

$F_{1,22}=1.10$, n.s.; Group x Region: $F_{1,22}=0.60$, n.s.), with both groups showing a greater percentage loss of cell bodies in the SNc than in the VTA ($F_{1,22}=19.13$, $p<0.001$). *Ad libitum* and food restricted rats also showed a similar percentage reduction in the optical density of the lesion striata (Figure 4.7F; $U=41.50$, n.s).

Histological data from the second dose response curve. As before, the percentage reduction in ipsilateral cell bodies was greater in the SNc than in the VTA (Figure 4.7C; Region: $F_{1,22}=30.94$, $p<0.001$). The percentage loss of SNc and VTA cell bodies was similar for *ad libitum* and food restricted rats (Region x Group: $F_{1,22}=0.67$, n.s.; Group: $F_{1,22}=0.43$, n.s.), who were also matched in terms of the percentage reduction of optical density in their lesion striata (Figure 4.7G; $U=49.50$, n.s).

4.3.3.2. First dose response curve

Because the five original groups (Figure 4.6) did not differ from each other in terms of their rotational bias (Group: $F_{4,19}=0.69$, n.s.), or in their performance on the adjusting step test (Group: $F_{4,19}=0.66$, n.s.), and the vibrissae test (Group: $F_{4,19}=0.37$, n.s.), data from all rats that had *ad libitum* or restricted food intake during the first dose response curve were pooled to create one *ad libitum* ($n=15$) and one food restricted ($n=9$) group and a second analysis conducted.

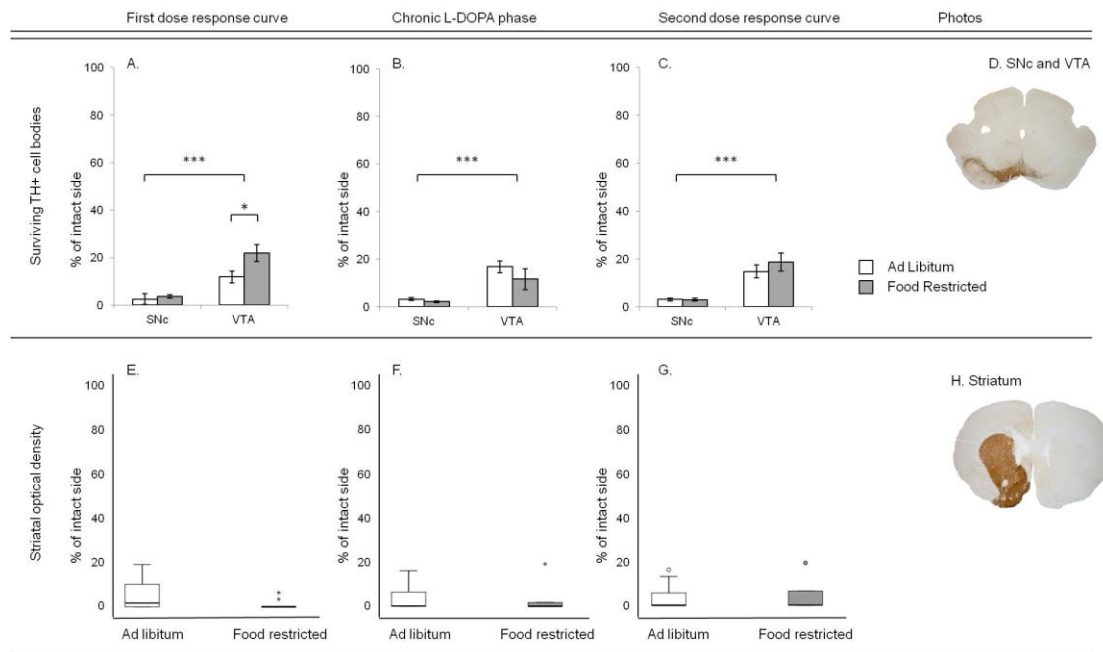


Figure 4.7. The surviving TH+ cell bodies in the SNc and VTA (top row) and the striatal optical density (bottom row), both expressed as a percentage of the intact side, in rats with *ad libitum* ($n=15/19/17$) and restricted ($n=9/5/7$) food access

during the first dose response curve (left column), the chronic L-DOPA treatment phase (middle column), and in the second dose response curve (right column). The figure also includes an illustrative example of the dopamine denervation observed in the SNc, VTA (D), and striatum (H). The bar charts show the mean, and the error bars show the standard error of the mean. The box plots show the median and interquartile ranges. Circles represent outliers. SNc=Substantia nigra pars compacta; VTA=Ventral tegmental area; TH=Tyrosine hydroxylase. *** $p < 0.001$.

Net rotations. Acute L-DOPA increased the contralateral bias of rats' rotations, with a similar rotational bias observed in the *ad libitum* and food restricted groups (Figure 4.8A; Dose: $F_{6,126}=4.27$, $p < 0.01$; Group: $F_{1,21}=0.30$, n.s.; Dose x Group: $F_{6,126}=1.52$, n.s.). While the groups' differed in the extent of their VTA denervation, this did not affect the net rotational bias to L-DOPA (Dose x VTA depletion: $F_{6,126}=0.41$, n.s.).

Adjusting step test. The food restricted group made more adjusting steps than the *ad libitum* group both when using their ipsilateral and contralateral forelimb (Figure 4.8C,E; Group: $F_{1,21}=11.01$, $p < 0.01$). However, despite food restricted rats making overall more adjusting steps, there was no significant difference in the relative contralateral deficit observed in the two groups, with all rats making relatively fewer adjusting steps with their contralateral than ipsilateral forelimb (Side: $F_{1,21}=52.41$, $p < 0.001$; Side x Group: $F_{1,21}=0.05$, n.s.). Acute administration of L-DOPA improved contralateral performance in both *ad libitum* and food restricted rats (Dose: $F_{6,126}=4.58$, $p < 0.05$; Dose x Side: $F_{1,126}=3.09$, $p < 0.01$; Simple Effect: $F_{6,16}=17.14$, $p < 0.001$; Dose x Group: $F_{1,126}=0.34$, n.s.). VTA denervation did not affect the contralateral deficit, nor did it affect the response to acute L-DOPA (Side x VTA: $F_{1,21}=0.14$, n.s.; Dose x VTA: $F_{6,126}=2.17$, n.s.)

Vibrissae test. Both the *ad libitum* and food restricted group exhibited a contralateral impairment in the vibrissae test (Figure 4.8G,I; Side: $F_{1,21}=88.01$, $p < 0.001$) that showed a modest improvement following acute L-DOPA administration (Dose: $F_{6,126}=2.77$, $p < 0.05$ Dose x Side: $F_{1,126}=2.25$; Simple Effect: $\min.F_{1,21}=55.62$, $p < 0.001$). Neither food restriction nor VTA denervation affected the motor response to acute L-DOPA (Group: $F_{1,21}=0.32$, n.s.; Dose x Group: $F_{6,126}=1.23$, n.s.; VTA x Side: $F_{1,21}=0.10$, n.s.; VTA x Dose: $F_{6,126}=1.54$, n.s.).

4.3.3.3. Second dose response curve

Because the five original groups (Figure 4.6) did not differ from each other in terms of rotational bias (Group: $F_{4,19}=0.13$, n.s.), the adjusting step test (Group: $F_{4,19}=0.79$, n.s.), and the vibrissae test (Group: $F_{4,19}=1.54$, n.s.), data from all rats that had *ad libitum* or restricted food intake during the second dose response curve were pooled to create one *ad libitum* (n=19) and one food restricted (n=5) group. Because there was no difference in the food restricted and *ad libitum* groups' VTA depletion during the second dose response curve this was not used as a covariate in the analysis below.

Net rotations. The groups continued showing an increase in the contralateral bias of their net rotations following acute L-DOPA administration. The net rotational bias was not affected by food restriction (Figure 4.8B; Group: $F_{1,22}=0.02$, n.s.; Dose: $F_{6,132}=22.21$, $p<0.001$; Group x Dose: $F_{6,132}=0.50$, n.s.).

Adjusting step test. The contralateral deficit in the adjusting step test was still evident following chronic L-DOPA treatment (Figure 4.8D,F; Side: $F_{1,22}=71.95$, $p<0.001$), but was alleviated by acute L-DOPA administration (Dose: $F_{6,132}=7.56$, $p<0.001$; Side x Dose: $F_{6,132}=34.14$, $p<0.001$; Effect of Dose on Side: $F_{6,17}=37.03$, $p<0.001$). There was no effect of food restriction on rats' motor response to acute L-DOPA (Group: $F_{1,22}=3.67$, n.s.; Group x Dose: $F_{6,132}=0.65$, n.s.).

Vibrissae test. The contralateral deficit in the vibrissae test persisted after chronic L-DOPA treatment (Figure 4.8H,J; Side: $F_{1,22}=81.59$, $p<0.001$). Both the *ad libitum* and food restricted rats showed improvement of their contralateral vibrissae reflex following acute L-DOPA administration (Dose: $F_{6,132}=4.21$, $p<0.01$; Side x Dose: $F_{6,132}=5.84$, $p<0.001$; Simple Effect of Dose on Side: $F_{6,17}=4.36$, $p<0.05$). The motor response to acute L-DOPA was not affected by food restriction (Group: $F_{1,22}=0.74$, n.s.).

4.3.3.4. The effect of food restriction on abnormal involuntary movements

The experiment was designed to allow a comparison between the AIMs of rats that had (i) never been food restricted, (ii) been previously food restricted in the absence of L-DOPA, or (iii) previously been food restricted while administered L-DOPA. As illustrated in Figure 4.6, there was no difference in the treatment received by the 'FR control' and the 'FR 1' groups at the point of chronic L-DOPA treatment. Similarly, at the same time point, there was no difference between the treatment received by the 'FR interval' and the 'FR interval +2' group. Because the experience of the 'FR control' and the 'FR 1' groups were identical the time of chronic L-DOPA treatment

The Effect of Food Restriction on the Motor Response to Acute L-DOPA

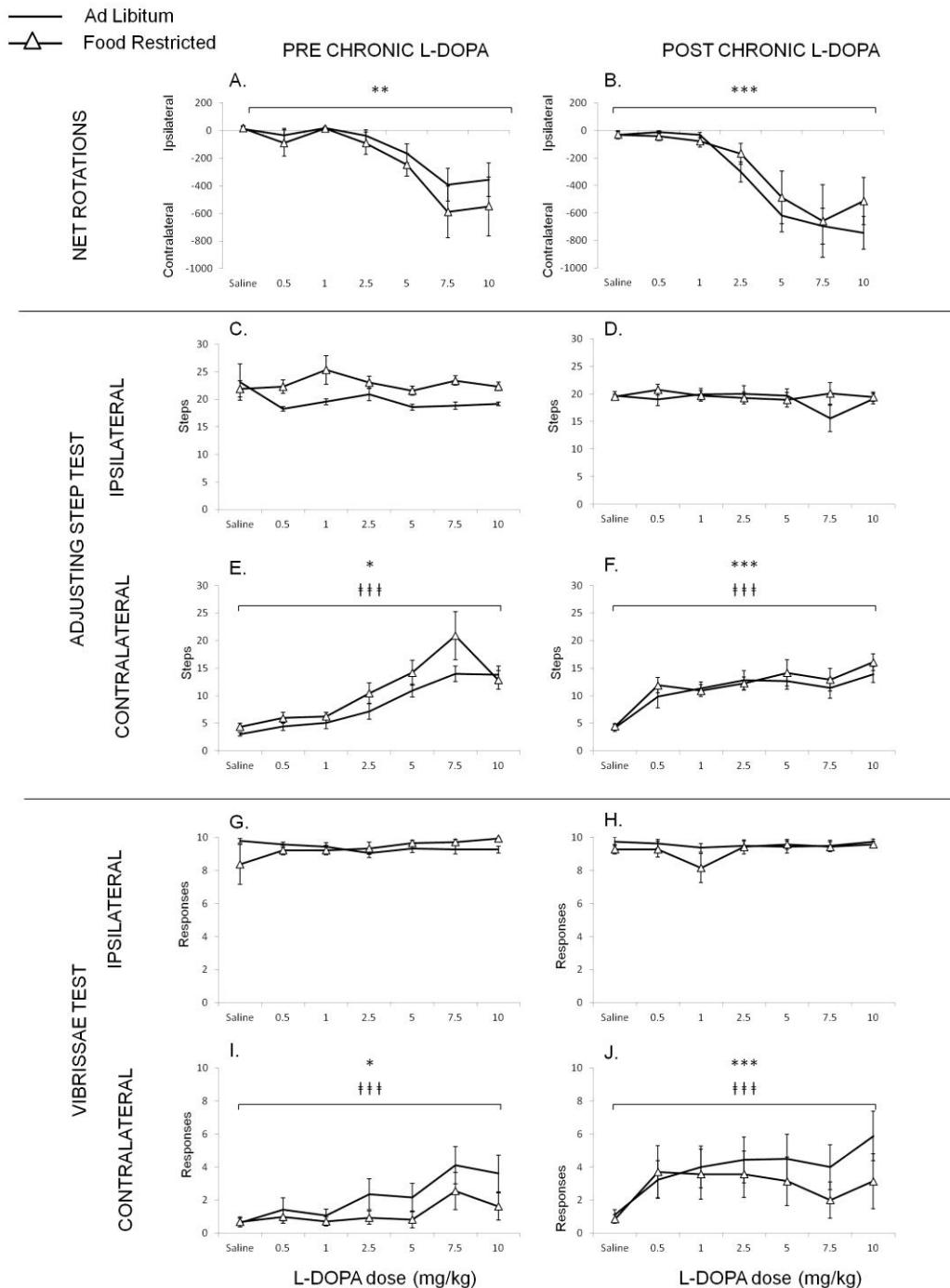


Figure 4.8. The motor effect of acute L-DOPA on net rotational bias (A-B), the adjusting step test (C-F), and the vibrissae (G-J) test on rats with MFB lesions that had *ad libitum* (n=15) or restricted (n=9) access to food. The dose response curves were generated before (left panel) and after (right panel) chronic L-DOPA treatment. The line charts show the mean, and the error bars show the standard error of the mean. MFB=Medial forebrain bundle. †Effect of Lesion, *Effect of L-DOPA; †p<0.05, *p<0.05, ††p<0.01, **p<0.01, †††p<0.001, ***p<0.001

and their AIMs scores did not differ (Group: $F_{4,23}=2.80$, n.s.), their data were pooled when analysing AIMs scores. The same was true for the ‘FR interval’ and the ‘FR interval +2’ groups.

Analysis following pooling showed that there was no difference in AIMs of rats that had never been food restricted (*ad libitum* controls), that had been food restricted during the first dose response curve (the ‘FR control’ and ‘FR1’ groups), or that been food restricted in the absence of L-DOPA (the ‘FR interval’ and the ‘FR interval +2’ groups; Figure 4.9A; Group: $F_{2,21}=0.74$, n.s.). Nor was there a difference between the groups when comparing the change in their AIMs magnitude in the chronic L-DOPA phase (Figure 4.9B; Group: $F_{1,21}=0.37$, n.s., Day x Group: $F_{12,126}=0.63$, n.s.).

4.3.3.5. The effect of chronic L-DOPA treatment on the motor response to acute L-DOPA

To test whether chronic L-DOPA treatment increased lesion rats’ motor response to subsequent acute L-DOPA, the motor data from the first and second dose response curve were compared. In the following section data from all rats were analysed together.

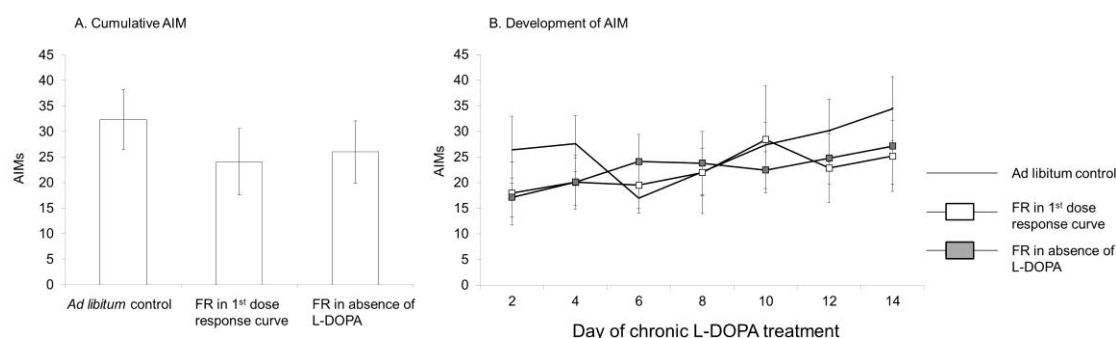


Figure 4.9. The final AIMs scores (A) and the development of the AIMs (B) in the *ad libitum* control group (n=9), and rats that were food restricted during the first dose response curve (n=9; FR control & FR+1), and rats that were restricted between the two dose response curves (n=6; FR interval & FR+2). The bar charts and line charts show the mean, and the error bars show the standard error of the mean. Ad lib=*Ad libitum*; AIMs=Abnormal involuntary movements, FR=Food restriction.

Net rotations. After chronic L-DOPA treatment, rats showed a greater net contralateral rotational bias following acute administration of L-DOPA doses 1.0, 2.5,

5.0, 7.5 and 10.0 mg/kg (Figure 4.10A; Chronic L-DOPA: $F_{1,22}=22.49$, $p<0.001$, Chronic L-DOPA x Dose: $F_{6,132}=4.72$, $p<0.001$; Simple Effect: $\min.F_{1,22}= 4.71$, $p<0.05$).

Adjusting step test. After chronic L-DOPA treatment, there was a significant increase in the number of contralateral steps made following acute administration of 1.0, 2.5, 7.5, and 10.0 mg/kg L-DOPA, as well as a small but significant decrease in the number of ipsilateral steps made following acute administration of 0.5 and 10.0 mg/kg L-DOPA (Figure 4.10B,C; Chronic L-DOPA: $F_{1,23}=0.13$, n.s.; Chronic L-DOPA x Dose x Side: $F_{6,138}=10.24$, $p<0.001$; Simple Effect: $\min.F_{1,23}= 6.76$, $p<0.01$).

Vibrissae test. Chronic L-DOPA treatment significantly increased the number of contralateral responses made in response to vibrissae stimulation following acute administration of 0.5, 1.0, 2.5, and 5.0 mg/kg L-DOPA (Figure 4.10D,E; Chronic L-DOPA: $F_{1,23}=10.08$, $p<0.01$; Chronic L-DOPA x Side x Dose: $F_{6,138}=2.20$, $p<0.05$; Simple Effect: $\min.F_{1,23}= 6.55$, $p<0.05$).

4.4. Discussion

L-DOPA competes with dietary amino acids for LAT1 mediated transport across the BBB (del Amo *et al*, 2008; Kageyama *et al*. 2000) and the presence of dietary amino acids has been shown to significantly decrease the amount of L-DOPA accessing LAT1 transport (Kageyama *et al*, 2000). It was therefore hypothesised that food restriction would increase the amount of L-DOPA crossing the BBB in food restricted relative to *ad libitum* rats, and that this would result in a greater motor response to acute L-DOPA in food restricted rats. If true, this would have implications for the dose of L-DOPA that was chosen for acute administration in later experiments where the experimental paradigm necessitated that rats were food restricted during testing.

Later thesis project experiments also planned to test the behavioural effect of acute L-DOPA in rats that had previously received chronic L-DOPA treatment. The behavioural response to acute L-DOPA depends not only on the amount of L-DOPA that crosses the BBB, but also on the sensitivity of striatal dopamine receptors. Following LID onset these receptors become sensitized (Konradi *et al*, 2004) and the

The Effect of Chronic L-DOPA on the Motor Response to acute L-DOPA Administration

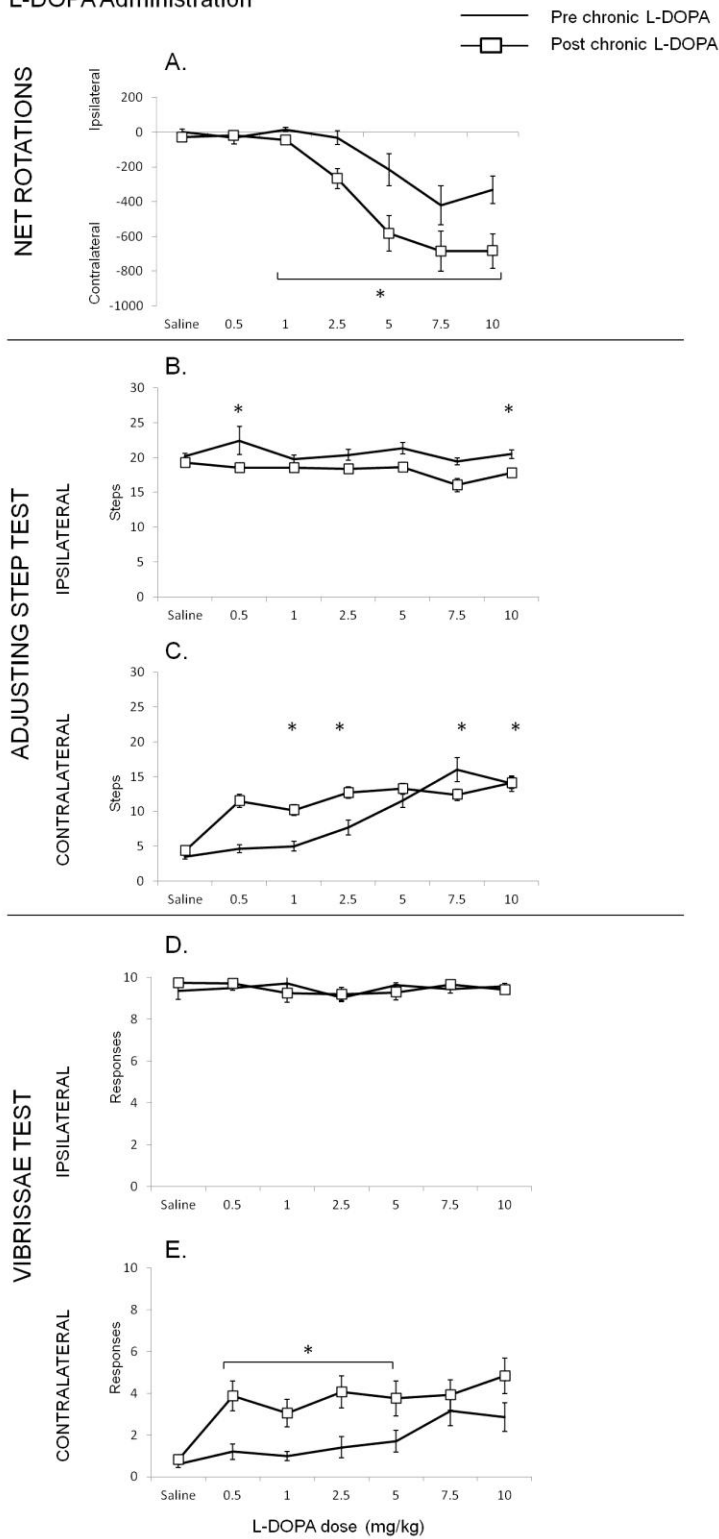


Figure 4.10. The effect of chronic L-DOPA treatment on the MFB lesion groups' (n=24) net rotational bias (A), adjusting step test (B-C), and vibrissae test (E-F) performance. The line graphs show the acute motor response to the six L-DOPA doses before and after chronic L-DOPA treatment. The line graph shows the mean, and the error bars the standard error of the mean.* p<0.05.

rotational response to L-DOPA increase (Henry *et al* 1998). Therefore, it was hypothesised that rats that had been chronically treated with L-DOPA would show a greater motor response to acute L-DOPA than rats with little or no previous L-DOPA exposure. The hypotheses were tested both in rats with unilateral intra-striatal and MFB lesions.

4.4.1. The effect of food restriction

There was an increased motor response to acute L-DOPA following food restriction in the intra-striatal lesion model. The effect was most evident when comparing the *ad libitum* and food restricted groups' net rotational bias. However, it is worth noting that this significant group difference was driven by a contralateral net bias in the food restricted group and a near absence of rotations in the *ad libitum* group. It is also worth noting that the contralateral rotational bias in the food restricted group was not greater at 10.0 mg/kg than at 5 mg/kg L-DOPA as would have been expected. The latter could be related to the occurrence of AIMs; AIMs magnitude increase following administration of higher L-DOPA doses (Winkler *et al*, 2002) and personal observations suggest that rats rotate less when exhibiting high forelimb and axial AIMs. No robust effect of food restriction was observed in the intra-striatal cohort when analysing data from the adjusting step and vibrissae tests. It is possible that the modest contralateral deficit observed in these tests caused a ceiling effect, thus making these tests less sensitive to detecting L-DOPA mediated improvements of motor function in the intra-striatal lesion model.

Unexpectedly, there was no effect of food restriction on the motor response to L-DOPA in the MFB model. In the intra-striatal model, the effect of acute L-DOPA on motor function had been most evident when measuring the rats' rotational bias. When discussing the absence of an effect of food restriction in the MFB model it is therefore worth noting that, unlike the intra-striatal lesion rats, both the *ad libitum* and food restricted MFB lesion rats showed a contralateral rotational bias following acute L-DOPA administration (Figures 4.3 and 4.8). A rotational response to L-DOPA requires denervation of not only the SNc but also the VTA (Thomas *et al*, 1994). The MFB model, which causes greater denervation in both of these structures than intra-striatal lesions (e.g. Deumens, 2002; Yuan *et al*, 2005) may therefore be more sensitive to L-DOPA-induced rotations. I therefore hypothesised that the greater

striatal and VTA dopamine denervation in the MFB lesion model contributed to a ceiling effect which masked the effect of food restriction in this model.

In all, the data from the intra-striatal experiment supported the hypothesis that food restriction increases transport of L-DOPA across the BBB, and that this results in an increased motor response to acute L-DOPA. While data from the MFB model did not support the hypothesis it is possible that an effect of food restriction on motor behaviour was masked by a ceiling effect, caused by the extensive dopamine denervation that occurs following MFB lesions. On a practical level, the findings suggest that the dose of acute L-DOPA administered to rats in order to achieve a therapeutic effect without overdosing them needs to be adjusted following food restriction in the intra-striatal but not in the MFB lesion model.

4.4.2. Abnormal Involuntary Movements

Unexpectedly, the data from the intra-striatal lesion model showed higher AIMs scores in rats that had been food restricted during the first L-DOPA dose response curve. This was in spite of the fact that all rats were allowed *ad libitum* access to food during chronic L-DOPA treatment. The development of LID is believed to depend on stimulation of D1 type receptors (e.g. Pearce *et al*, 1998). Imaging experiments have shown that, in the rat 6-OHDA MFB model, as few as seven injections of a D1 agonist lead to an increased striatal blood-oxygen-level dependent (BOLD) signal following subsequent D1 agonist exposure and that this signal correlates with at least forelimb AIMs (Delfino *et al*, 2007). These data suggest that D1 stimulation underlies not only the development of LID, but also the acute expression of AIMs. It can therefore be hypothesised that the greater AIMs observed in the intra-striatal lesion group that was food restricted during initial L-DOPA exposure was driven by greater D1 sensitization in this, relative to the *ad libitum* control, group.

In addition to higher AIMs, the food restricted intra-striatal lesion group also showed greater contralateral turning in response to acute L-DOPA than the *ad libitum* group. Contralateral turning in at least MFB lesion rats is driven by synergistic stimulation of D1 and D2 receptors (Paul *et al*, 1992). Similar to the effect of D1 agonist stimulation on AIMs (Delfino *et al*, 2007), repeated D1 agonism increases the rotational response to dopamine agonist administration in lesion rats (Delfino *et al*, 2004). While the greater contralateral net bias observed in the intra-striatal food restricted relative to the *ad libitum* group was originally hypothesised to be driven by

greater L-DOPA influx, it could therefore also be hypothesised that it was linked to increased D1 sensitization in the food restricted relative to the *ad libitum* group. Supporting the hypothesis are previously published findings showing that food restricting rats to 80% of their baseline weight increases the motor activity induced by acute D1 agonist administration (Carr *et al*, 2003). To further test this hypothesis, future experiments may wish to measure D1 receptor sensitivity following food restriction. Such assays were, however, beyond the scope of the current experiment and therefore not conducted as part of the experiments presented in this chapter.

One limitation of the intra-striatal lesion experiment was that it was unclear whether an effect of food restriction on behaviour was due to food restriction alone, or due to an interaction between food restriction and L-DOPA treatment. While the primary aim of the MFB experiment was to test the effect of food restriction and chronic L-DOPA on the motor response to acute L-DOPA administration, the experiment also included additional experimental groups to allow exploration of unanswered questions from the intra-striatal experiment. To this end, the MFB lesion experiment compared the AIMs scores exhibited by rats that had (i) never been food restricted, (ii) been food restricted in the absence of drugs, or (iii) been food restricted while initially exposed to L-DOPA. However, no effect of food restriction on subsequent AIMs was found in the MFB model.

As previously stated, MFB lesions cause a more extensive dopamine loss than intra-striatal lesions. Previously published studies have demonstrated that greater dopamine loss increases both the magnitude of AIMs scores, and the proportion of rats that develop dyskinesia following chronic L-DOPA treatment (Winkler *et al*, 2002). Furthermore, dopamine activated striatal adenylyl cyclase activity, which is linked to stimulation of D1 receptor which in turn are implicated in LID, is higher in tissue from rats with complete rather than partial (57% loss) dopamine denervation (Piffl *et al*, 1992), which would mimic the MFB over the intra-striatal model. It is thus possible that a potential effect of food restriction on AIMs magnitude, if linked to D1 receptor sensitisation, may have been masked by a ceiling effect in the MFB lesion model which is characterised by a more extensive dopamine loss, as well as greater AIMs (Winkler *et al*, 2002).

When discussing the potential effect of food restriction on AIMs it should be noted that there are currently no tests available to accurately predict the susceptibility to LID and magnitude of subsequent AIMs. Therefore, before firm conclusions can be

drawn from the intra-striatal experiment, the data should be replicated to ensure the validity of the findings. Ideally, such experiment would use the design of the MFB lesion experiment to allow dissociation between the effect of food restriction in the absence of L-DOPA versus the effect of food restriction in conjunction with L-DOPA on later AIMs.

4.4.3. The Effects of Chronic L-DOPA

Chronic L-DOPA has previously been shown to sensitize D1 receptors in lesion rats (Konradi *et al*, 2004), increase the rotational response to L-DOPA (Henry *et al*, 1998), and improve performance on simple motor tests (Winkler *et al*, 2002). Experiments 3 and 4 therefore tested the effect of chronic L-DOPA treatment on the motor response to acute L-DOPA, as measured by rotometers, the adjusting step test, and the vibrissae test.

In the intra-striatal experiment an increased contralateral motor response to acute L-DOPA following chronic treatment was only observed in the vibrissae test. The small magnitude of this effect and the absence of an altered contralateral response on the other motor tests suggest that the effect of chronic L-DOPA on the motor response to subsequent L-DOPA is minimal in this model.

In the MFB model, chronic L-DOPA treatment increased the motor response to acute L-DOPA administration on all three tests of motor function. The data therefore confirmed previous reports of an effect of chronic L-DOPA on the adjusting step test (Winkler *et al*, 2002) and net rotational bias (Henry *et al*, 1998) whilst extending previous findings by also showing an effect on the vibrissae test.

The different effects of chronic L-DOPA in the two models are hypothesised to relate to the extent of dopamine loss the two lesions induce. MFB lesions cause more extensive denervation in both the SNc and VTA than intra-striatal lesions. As previously stated, VTA denervation increases the rotational response to L-DOPA (Thomas *et al*, 1994). Hence, the difference in the two models' rotational response to L-DOPA is hypothesised to be caused by the greater extra-striatal dopamine loss in the MFB relative to the intra-striatal lesion model. Furthermore, the difference in the observed effect of chronic L-DOPA on the adjusting step is hypothesised to relate to the greater baseline deficit observed on this test in the MFB, relative to the intra-striatal, lesion model.

4.4.4. Conclusions

The data provided partial support for the hypotheses that restricted food intake and chronic L-DOPA exposure both increase the motor response to acute L-DOPA, whilst also highlighting vital differences between two commonly used preclinical models of PD. The less severe intra-striatal lesion model exhibited a modest motor response to acute L-DOPA that was increased in the food restricted group. Conversely, the MFB model showed greater baseline motor deficits and heightened motor response to acute L-DOPA following chronic treatment but not food restriction.

The differences between the two lesion models have methodological implications for preclinical experiments involving acute L-DOPA administration to lesion rats following food restriction or chronic L-DOPA treatment. When administering acute L-DOPA to rats with MFB lesions the dose needs to be decreased if the therapeutic effect is to be similar in rats that have received long-term L-DOPA treatment and rats that are drug naïve. When acute L-DOPA is administered to rats with intra-striatal lesions, the dose needs to be decreased if a similar therapeutic effect is to be observed in rats on food restriction relative to rats with *ad libitum* access to food.

While the data provided partial support for the experimental hypothesis that food restriction increases L-DOPA influx, it is noted that the current chapter only measured the behavioural output of L-DOPA administration and food restriction. As such, the mechanism underlying the observed effect of food restriction on the motor response to L-DOPA in the intra-striatal model was not directly tested. To test the hypothesis that food restriction increased the rotational response to acute L-DOPA in the intra-striatal model by increasing the influx of L-DOPA, Chapter 5 used microdialysis to measure the striatal extracellular DOPA levels before and after acute L-DOPA administration in rats with free or restricted food intake.

5. Investigating the Effect of Food restriction on Striatal L-DOPA Influx

Background: The experiments reported in Chapter 4 revealed evidence of an increased rotational response to acute L-DOPA following food restriction in the intra-striatal 6-OHDA lesion model of PD. This was hypothesized to be caused by increased L-DOPA influx via the BBB in food restricted versus *ad libitum* rats.

Chapter aim: The aim of Experiment 5 was to test whether food restriction increases influx of L-DOPA in rats with 6-OHDA lesions to the striatum or MFB.

Methods: The hypothesis was tested in both the intra-striatal and MFB 6-OHDA model. Bilateral microdialysis was used to measure striatal DOPA levels in freely moving rats with restricted or *ad libitum* access to food before, and after, acute L-DOPA administration.

Results: Food restriction did not affect the striatal DOPA levels observed following acute L-DOPA administration in the intra-striatal or MFB lesion model. Therefore, the hypothesis was not supported. Food restriction did, however, decrease baseline dopamine levels. In addition, food restriction increased 5HIAA levels in the intra-striatal, but not the MFB model.

5.1 Introduction

Chapter 4 described an increased rotational response to acute L-DOPA following food restriction of rats with intra-striatal 6-OHDA lesions. L-DOPA is known to compete with dietary amino acids for transport across the BBB (reviewed in del Amo *et al*, 2008). It was therefore hypothesized that food restriction, by decreasing competition for LAT1-mediated transport, indirectly increased the amount of L-DOPA that crossed the BBB. Ultimately, this was hypothesized to have caused higher dopamine levels in the striata of food restricted rats relative to *ad libitum* rats, leading to a greater motor response to acute administration of L-DOPA. This is in line with the recognition that food intake affects L-DOPA response in the PD patients. In the clinic, this recognition has led to recommendations that patients experiencing LID or “off periods” should redistribute protein intake so as to consume less protein during breakfast and lunch (Olanow *et al*, 2001; Cereda *et al*, 2010). The rationale behind the suggestion is that in advanced PD, when there is reduced striatal dopamine storage capacity, striatal dopamine levels are highly sensitive to small changes in the L-DOPA transport into the brain (Olanow *et al*, 2001). Hence, by decreasing protein intake, and thus increasing the amount of L-DOPA that accesses transport across the BBB, it is believed that the motor response to L-DOPA can be enhanced (Olanow *et al*, 2001).

Based on current literature, it was hypothesized that the increased rotational response to L-DOPA observed following food restriction in the intra-striatal model (Experiment 3) was due to greater influx of L-DOPA. However, food restriction may also affect the motor response to L-DOPA through other routes. For example, food restriction increases the motor response to D1 agonists (Carr *et al*, 2003) and one year exposure to a low protein diet reduces dopamine clearance in 6-OHDA lesion rats (Sevak *et al*, 2008). To further determine the mechanism underlying the phenomenon observed in Chapter 4, the hypothesis that the increased motor response to L-DOPA observed in food restricted intra-striatal lesion rats was mediated by an increase in the L-DOPA that was transported over the BBB was tested in the current chapter using microdialysis in freely moving lesion rats with *ad libitum* or restricted access to food. Using a microdialysis paradigm, extracellular levels of DOPA, DOPAC, HVA, dopamine, and 5HIAA in dorsolateral striatum were measured before and after acute s.c. administration of L-DOPA were measured. DOPA levels were of most interest as

they allowed direct testing of the hypothesis that food restriction increases the amount of L-DOPA that crosses the blood brain barrier. Dopamine, DOPAC, HVA levels were measured to as these are indicative of the impact L-DOPA has on dopamine and therefore also the motor response to the drug. In addition, the DOPAC/dopamine ratio was calculated as an estimate of dopamine utilization (e.g. Cartmell *et al*, 2000; Jentsch *et al*, 1998). 5HIAA was used as a control substrate to demonstrate that a potential increase in the neurotransmitter concentrations measured post L-DOPA injection was specific to DOPA, dopamine and its metabolites rather than reflective of a non-specific effect on both dopaminergic and non-dopaminergic systems. It was thus hypothesised that 5HIAA levels would remain constant after acute L-DOPA injection while levels of DOPA, dopamine, DOPAC, and HVA would increase. To ensure consistency with Chapter 4, the hypothesis was tested in rats with sham or 6-OHDA lesions to the striatum or MFB.

5.1.2. Aims

The aim of the experiment was to test the hypothesis that reduced food intake increases the influx of L-DOPA to the striatum. The hypothesis was tested by measuring extracellular levels of DOPA in the dorsolateral striatum before and after acute L-DOPA administration using microdialysis in freely moving rats with sham or 6-OHDA lesions to the MFB or striatum. Half of the rats in each experimental group were food restricted while the remaining half was kept as *ad libitum* controls.

5.2. Methods

5.2.1. Experimental Design

Rats were given 6-OHDA or sham lesions to the striatum or MFB. Rats with 6-OHDA lesions were allocated to an *ad libitum* or food restricted group based on their amphetamine induced rotations. After one week of restricted or free access to chow, rats underwent microdialysis probe surgery. Following a 48 h recovery period, extracellular striatal levels of L-DOPA, dopamine, DOPAC, HVA, and 5HIAA were measured before and after acute administration of 5 mg/kg L-DOPA. Figure 5.1 contains a graphical representation of the procedures each rat underwent. Please note that, for practical reasons, a limited number of rats could be tested at one time. Therefore, animal orders, lesions, probe implants, and dialysis were staggered and the experiment conducted over a 4 month period

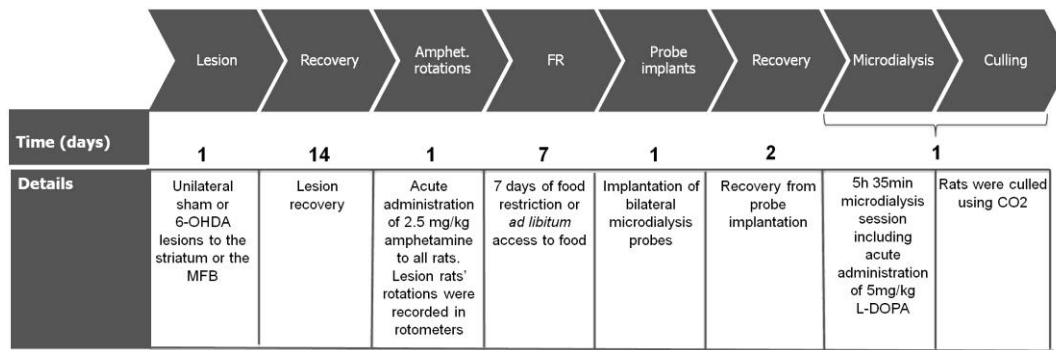


Figure 5.1. A graphical representation of the procedures each rat in Experiment 5 underwent. Please note that the experiment included eight batches of rats, which underwent their lesions, implants, and dialysis at the same time. For practical reasons, the testing or each group was staggered causing the total experiment to span over 11 weeks. Amphet=amphetamine, FR=Food restriction, MFB=Medial forebrain bundle.

5.2.2. Procedure

Female Sprague Dawley rats were randomly allocated to one of four surgery groups and given unilateral sham MFB, 6-OHDA MFB, sham intra-striatal, or 6-OHDA intra-striatal lesions. MFB and intra-striatal lesions were induced using the previously described lesion protocol (sections 2.2.2 and 2.2.3). Sham controls received an infusion of PBS at the same rate and in the same coordinates as their 6-OHDA counterparts. The net ipsilateral bias of 6-OHDA lesion rats' amphetamine induced rotations (section 2.4.6.2) was used to allocate them to food restricted and *ad libitum* groups. Amphetamine-induced rotations were used over other motor tests to ensure consistency with Chapter 4, which the current experiment was based on. For the same reason, Sprague Dawley rats were used instead of the Lister Hooded strain utilised in the experiments presented in Chapters 6 to 8.

Eight experimental groups were included in the experiment: sham MFB *ad libitum* (n=6); sham MFB food restricted (n=7); 6-OHDA MFB *ad libitum* (6); 6-OHDA MFB food restricted (n=8); sham intra-striatal *ad libitum* (n=7); sham intra-striatal food restricted (n=6); 6-OHDA intra-striatal *ad libitum* (n=5); 6-OHDA intra-striatal food restricted (n=6).

Following lesion recovery and amphetamine-induced rotation testing, rats were allowed one week of free or restricted access to food. They thereafter underwent probe implantation surgery (2.6.1) and, following a 48 h recovery session, a micordialysis session was conducted. The dialysis session spanned over 5 h and 35

min, as further described in section 2.6.2. Microdialysis samples were taken every 15 min, and immediately stored at -20°C before being moved to -80°C at the end of testing. Two hours and 15 min after the first dialysis sample was taken, the rats were given one s.c. injection of L-DOPA at a concentration of 5 mg/kg and a volume of 1 ml/kg. Sampling thereafter continued until the end of the microdialysis session, when rats were euthanized using CO². While other experiments reported in this thesis used Euthatal for euthanizing rats, CO² was used in the current experiment as it was conducted in another facility (Eli Lilly & Co Ltd, Windlesham, Surrey) and therefore also under another project licence. In instances where one probe became blocked or damaged prior to microdialysis testing, unilateral sampling was conducted. The number of rats in each group from whom ipsilateral and contralateral samples were collected is specified in Table 5.1.

The L-DOPA dose used (5 mg/kg) had previously been shown to produce a motor response to L-DOPA in the MFB and intra-striatal lesion models (Chapter 4) and was as such considered a valid choice. Furthermore, in a pilot study including two lesion rats (data not shown), the AIMs and L-DOPA induced rotations observed in MFB lesion rats following acute administration of 10 mg/kg L-DOPA interfered with the microdialysis set-up and increased the risk of harm to the rats. Specifically, when exhibiting AIMs of high magnitude, rats risked becoming entangled in the tubing which posed a risk of injury. In addition, entangling and twisting of the tubes disrupted the flow of aCSF. Decreasing the dose of acute L-DOPA administered to lesion rats is known to reduce the magnitude of their AIMs (e.g. Winkler *et al*, 2002). Using a L-DOPA dose of 5 mg/kg, rather than the 10 mg/kg used during chronic L-DOPA treatment in the other experiments reported in this thesis, was therefore preferable for animal welfare reason (reduced risk of rats becoming entangled in tubing) as well as for scientific reasons (reduced risk for disruption to the aCSF flow and lost microdialysis samples).

HPLC analysis of dialysis samples was conducted by Jane Cooper (Eli Lilly & Company, Windlesham, Surrey, UK) as described in 2.6.3. In instances where the HPLC was unable to detect the substance being measured or provided a read-out value less than 0.1, a value of 0.1 ng/ml was inserted in its place. This corresponds to the lowest level the HPLC could reliably measure and the approach is in accordance with the standard practise in the lab conducting the HPLC analysis.

Table 5.1. The table shows the number of rats in each experimental group from which microdialysis samples from the intact and lesion striata could be collected. Ad Lib = *ad libitum*. FR = food restricted, MFB=Medial forebrain bundle

| | MFB Sham Lesions | | MFB 6-OHDA Lesions | | Intra-striatal Sham Lesions | | Intra-striatal 6-OHDA Lesions | |
|--------|------------------|----------------|--------------------|----------------|-----------------------------|----------------|-------------------------------|----------------|
| | Intact Striata | Lesion Striata | Intact Striata | Lesion Striata | Intact Striata | Lesion Striata | Intact Striata | Lesion Striata |
| Ad Lib | 5 | 5 | 6 | 5 | 6 | 7 | 5 | 5 |
| FR | 6 | 7 | 8 | 7 | 6 | 6 | 6 | 5 |

5.2.3. Statistical analysis

The MFB and intra-striatal lesion rats were analysed separately to ensure consistency with the analysis of the dose-response curves in Chapter 4.

Amphetamine induced rotations were analysed using a one-way ANOVA. Baseline microdialysis levels were measured by inserting the mean concentration (ng/ml) from the five baseline samples into a repeated measures ANOVA where Side was used as a within subject variable, and Lesion and Food restriction were used as between-subject variables.

The effect of acute L-DOPA was measured in two ways. First, all 18 samples were analysed in a repeated measures ANOVA where Time and Side were used as within-subject variables, and Food restriction and Lesion used as between-subject variables. This analysis (i) provided information on the time course of neurochemical changes that occurred following L-DOPA injection, and (ii) took into consideration the difference in the groups' baseline levels. In the second analysis, the average post-injection levels was expressed as a percentage of baseline and analysed using a repeated measures ANOVA where Side was used as a within-subject variable, and Lesion and Food restriction were used as between-subject variables. This analysis tested whether the groups differed in the relative change of neurochemical levels following L-DOPA injection. The analysis supplemented the previous analysis by not taking baseline differences between the groups into consideration and instead comparing the groups in terms of their relative change in DOPA, dopamine, DOPAC, HVA, and 5HIAA levels. In both analyses, significant between-group differences were analysed using a post-hoc Scheffe test and significant interactions analysed using a test of simple effects.

5.3. Results

5.3.1. Amphetamine induced rotations

Two weeks post-lesion, all rats were given an acute amphetamine administration and the lesion rats' rotations were recorded using automated rotometers. The results were used to allocate 6-OHDA lesion rats to a food restricted or *ad libitum* group. All lesion rats showed a strong ipsilateral bias in their rotations (Figure 5.2). There was no difference in the net bias of rats that were later food restricted or kept as *ad libitum* controls in either the 6-OHDA MFB ($F_{1,14}=0.23$, n.s.) or the 6-OHDA intra-striatal ($F_{1,12}=0.00$, n.s.) cohort.

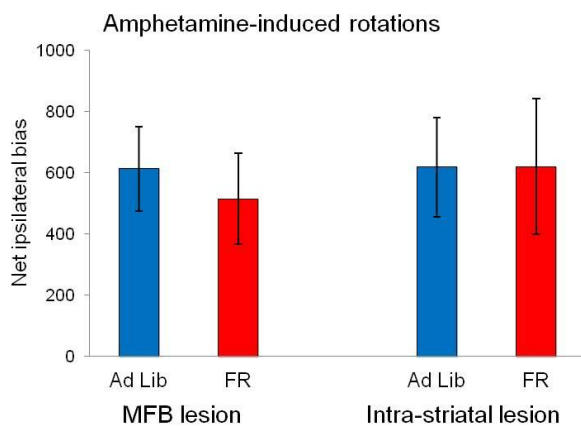


Figure 5.2. The net ipsilateral bias of the amphetamine induced rotations measured in rats with MFB or intra-striatal 6-OHDA lesions. All rotations were conducted prior to food restriction. "Ad Lib" and "FR" indicate later group allocation and not the food status at the time of amphetamine rotations. The bars show the mean bias and the error bars the standard error of the mean. Ad Lib=(subsequently) *ad libitum*, FR= (subsequently) food restricted, MFB=Medial forebrain bundle

5.3.2 Baseline measurements

5.3.2.1. DOPA

MFB model. There was a slight but significant asymmetry in the DOPA levels in the sham *ad libitum* group, with marginally higher DOPA levels observed in the intact than lesion striata. There was no difference between the amount of DOPA measured in the intact and lesion striata of the other groups (Figure 5.3A; Side x Lesion x Food restriction: $F_{1,24}=5.19$; Simple Effect of Side: $F_{1,24}=7.56$, $p<0.01$).

Intra-striatal model. The intra-striatal cohort showed an equal amount of DOPA in both the lesion and intact striata (Figure 5.3B; Side: $F_{1,20}=0.74$, n.s.; Side x Lesion:

$F_{1,20}=0.45$, n.s.). Neither the 6-OHDA lesion nor food restriction affected baseline DOPA levels (Lesion: $F_{1,20}=0.97$, n.s.; Food restriction: $F_{1,20}=2.40$, n.s.).

5.3.2.2. Dopamine

MFB model. The 6-OHDA lesion caused a marked asymmetry in baseline dopamine levels, with lower dopamine levels in the lesion striata (Figure 5.3C; Side x Lesion: $F_{1,24}=7.25$, $p<0.05$; Simple Effect of Side on 6-OHDA group: $F_{1,24}=17.20$, $p<0.001$). Food restriction did not affect baseline dopamine levels (Food restriction: $F_{1,24}=0.85$, n.s.).

Intra-striatal model. The 6-OHDA lesion decreased dopamine levels in the lesion striata (Figure 5.3D; Side x Lesion: $F_{1,20}=12.66$, $p<0.001$; Simple Effect of Lesion: $F_{1,20}=10.64$, $p<0.01$). In addition, there was a strong trend towards a reduction in baseline dopamine levels following food restriction (Food: $F_{1,20}=4.05$, $p=0.058$).

5.3.2.3. DOPAC

MFB model. 6-OHDA lesions significantly decreased DOPAC levels in the lesion striata (Figure 5.3E; Side x Lesion: $F_{1,24}=6.87$, $p<0.05$; Simple Effect of Lesion: $F_{1,24}=27.20$, $p<0.001$). Food restriction did not affect baseline DOPAC levels (Food restriction: $F_{1,24}=0.00$, n.s.).

Intra-striatal model. In the intra-striatal model, DOPAC levels decreased in both the sham and 6-OHDA groups' lesion striata (Figure 5.3F; Side: $F_{1,20}=22.94$, $p<0.001$), although a marginally greater decrease was observed in the 6-OHDA lesion group (Side x Lesion: $F_{1,20}=4.61$, $p<0.05$; Simple Effect of Side: $F_{1,20}=22.18$, $p<0.05$). Food restriction did not affect DOPAC levels (Food: $F_{1,20}=0.42$, n.s.).

5.3.2.4. HVA

MFB model. 6-OHDA lesions decreased HVA levels in the lesion striata (Figure 5.3G; Side x Lesion: $F_{1,24}=15.71$, $p<0.001$; Effect of Side on Group: $F_{1,24}=33.68$, $p<0.001$). Food restriction did not affect baseline HVA levels (Food restriction: $F_{1,24}=0.07$, n.s.).

Intra-striatal model. Reduced HVA levels in the lesion striata of 6-OHDA rats were also observed in the intra-striatal model (Figure 5.3H; Side x Lesion: $F_{1,20}=11.71$, $p<0.01$; Effect of Lesion on Side: $F_{1,21}=37.62$, $p<0.001$). Whilst not reaching statistical significance, there was a trend towards a further reduction in HVA levels following food restriction (Food restriction: $F_{1,21}=3.65$, $p=0.07$).

5.3.2.5. DOPAC / DA

MFB model. 6-OHDA lesions decreased dopamine utilization in the lesion as well as the intact striatum (Figure 5.3I; Lesion: $F_{1,24}=7.23$ $p<0.05$; Side: $F_{1,24}=1.70$, n.s.; Side x Lesion: $F_{1,24}=0.00$, n.s.). Food restriction did not affect baseline dopamine utilization (Food restriction: $F_{1,24}=0.02$, n.s.; Side x Food restriction: $F_{1,24}=0.28$, n.s.)

Intra-striatal model. Overall, both the sham and 6-OHDA lesion group's dopamine utilization was lower in the lesion than intact striata (Side: $F_{1,20}=9.15$, $p<0.01$). 6-OHDA lesions were associated with a further decrease in dopamine utilization (Figure 5.3J; Lesion: $F_{1,20}=17.18$, $p<0.001$). While visual inspection of the data suggested the decreased utilization in the 6-OHDA group's was greater in the lesion than intact striata this did not meet statistical significance (Side x Lesion: $F_{1,20}=2.77$, n.s.) due to high variability in the data. Food restriction was associated with marginally higher overall dopamine utilization, although the variation in the data suggest that this statistically significant difference should be interpreted with caution (Food restriction: $F_{1,20}=5.06$, $p<0.05$; Side x Food restriction: $F_{1,20}=0.24$, n.s.).

5.3.2.6. 5HIAA

MFB model. The 6-OHDA lesion group had significantly lower 5HIAA levels in the lesion striata than sham controls (Figure 5.3K; Side x Lesion: $F_{1,24}=13.05$, $p<0.01$; Effect of Side on 6-OHDA groups: $F_{1,24}=32.19$, $p<0.001$). There was also an effect of food restriction, which increased extracellular 5HIAA levels (Food restriction: $F_{1,24}=6.83$, $p<0.05$).

Intra-striatal model. In the intra-striatal cohort, the food restricted sham and the *ad libitum* 6-OHDA animals had lower 5HIAA levels in their ipsilateral relative to their intact striata (Figure 5.3L; Side x Lesion x Food restriction: $F_{1,20}=4.60$, $p<0.05$; Effect of Food restriction: $F_{1,20}=5.31$, $p<0.05$).

5.3.3. Effect of acute L-DOPA on DOPA levels

MFB model. All MFB groups showed a peak in their extracellular DOPA levels following L-DOPA injection (Figure 5.4A-B, D-E; Time: $F_{17,408}=55.71$, $p<0.001$). This was reflected in the higher than baseline DOPA levels observed in all MFB groups after L-DOPA injection (Figure 5.4C,F).

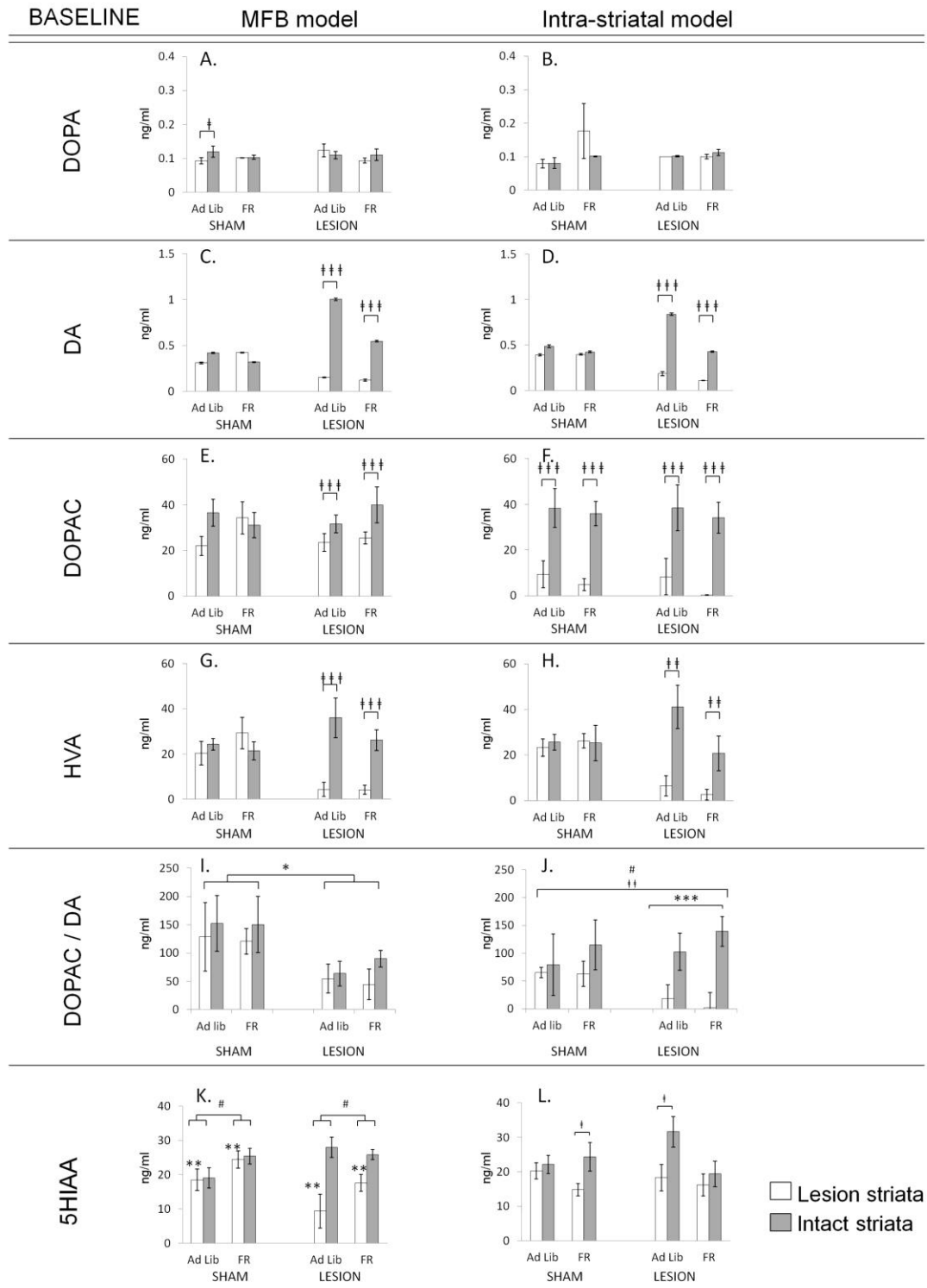


Figure 5.3. The average baseline levels of DOPA (A-B), dopamine, (C-D), DOPAC (E-F), HVA, (G-H), and 5HIAA (I-J) measured in rats with sham or 6-OHDA lesions to the MFB (left column) or striatum (right column). The error bars show the standard error of the mean. Ad lib=Ad libitum, FR=Food restriction. *Significant difference between sham and 6-OHDA lesion rats, #Significant difference between *ad libitum* and food restricted rats, †Significant difference intact and lesion, * $p < 0.05$, # $p < 0.05$, ** $p < 0.01$, † $p < 0.01$, *** $p < 0.001$

There was no significant difference between the extracellular DOPA levels measured in the sham and 6-OHDA lesion group when analysing all time points together (Lesion: $F_{1,24}=0.62$, n.s.; Side: $F_{1,24}=0.31$, n.s.; Side x Lesion: $F_{1,24}=3.19$, n.s), nor when measuring the post-injection levels expressed as a percentage of baseline (Lesion: $F_{1,24}=0.54$, n.s.; Side x Lesion: $F_{1,24}=3.13$, n.s.).

Neither did food restriction affect extracellular DOPA levels. This was true both when analysing all time points together (Food restriction: $F_{1,24}=0.99$, n.s.), and when comparing the groups' post-injection levels expressed as a percentage of baseline (Food restriction: $F_{1,24}=0.01$, n.s.; Side x Food restriction: $F_{1,24}=0.09$, n.s.).

Intra-striatal model. All intra-striatal groups showed a peak in their extracellular DOPA levels after L-DOPA injection (Figure 5.4G-H,J-K; Time: $F_{17,340}=19.46$, $p<0.001$). This was reflected in the higher than baseline DOPA levels observed in all groups post-injection (Figure 5.4I,L).

There was no significant difference between the DOPA levels observed in the sham and 6-OHDA lesion groups when including all time points in the analysis (Lesion: $F_{1,20}=0.18$, n.s.), nor when comparing the average post-injection DOPA levels expressed as a percentage of baseline (Lesion: $F_{1,20}=0.14$, n.s.).

While visual inspection of the data suggested higher absolute DOPA levels in the food restricted 6-OHDA lesion group, this was associated with a greater variation in their data and did not meet statistical significance when including all time point in the analysis (Food restriction: $F_{1,20}=1.60$, n.s.; Time x Food: $F_{17,340}=0.65$, n.s.), nor when only comparing average pre- and post-injection DOPA levels (Food restriction: $F_{1,20}=0.00$, n.s.; Side x Food restriction: $F_{1,20}=0.00$, n.s.).

5.3.4. Effect of acute L-DOPA on dopamine levels

MFB model. There was no notable increase or peak in extracellular dopamine levels following L-DOPA injection in the sham lesion group (Figure 5.5A-B). This was confirmed by data showing that their post-injection dopamine levels were averaging at approximately 100% of baseline levels (Figure 5.5C). The *ad libitum* 6-OHDA lesion group exhibited significant fluctuations in their extracellular dopamine levels over the course of testing (Figure 5.5D-E), a phenomenon which was not observed in the 6-OHDA lesion food restricted group (Time x Lesion x Food: $F_{17,408}=1.92$, $p<0.05$; Effect of Time on 6-OHDA *ad libitum* group: $F_{8,17}=4.10$, $p<0.05$).

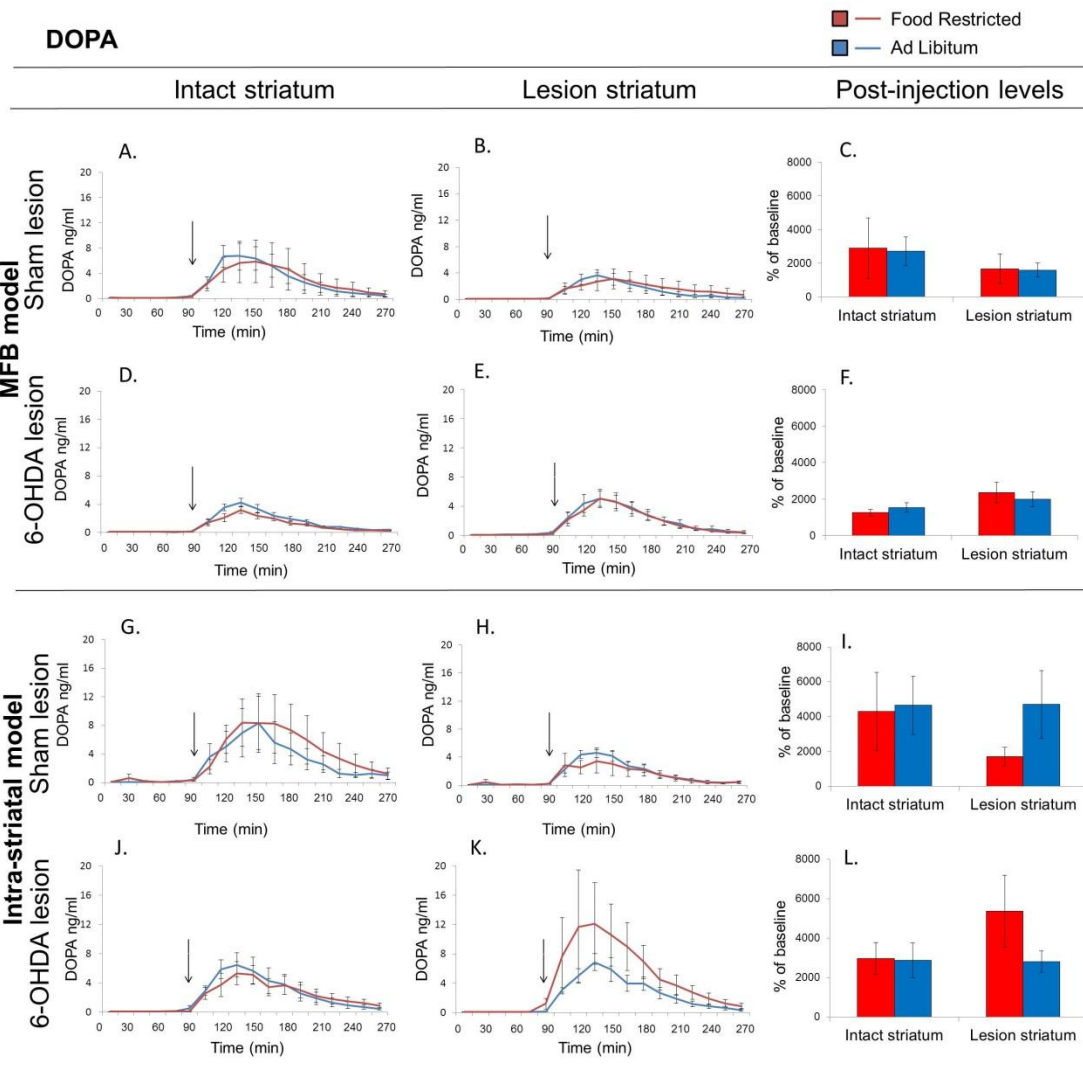


Figure 5.4. The DOPA levels in the intact and lesion striata of rats with sham or 6-OHDA lesions to the MFB (A-F) or the striatum (G-L) that experienced free or restricted access to food. The arrow represents the time point at which rats were administered 5 mg/kg L-DOPA. The column furthest to the right shows the mean post-injection DOPA levels relative to baseline, with 100% corresponding to baseline levels. The error bars show the standard error of the mean. MFB=Medial forebrain bundle.

However, visual inspection of the data and the finding that the lesion groups did not differ when comparing their average post-injection dopamine levels expressed as a percentage of baseline (Figure 5.5F; Lesion: $F_{1,24}=1.78$, n.s.; Side x Lesion: $F_{1,24}=2.49$, n.s.) suggested that the changes were driven by fluctuations in the data rather than an actual increase in dopamine levels.

Reflecting the success of the lesion, 6-OHDA lesion rats showed significantly decreased extracellular dopamine levels in the lesion, relative to the intact, striatum when including all time points in the analysis (Figure 5.5F; Side x Lesion; $F_{1,24}=7.92$, $p<0.05$; Effect of Side on 6-OHDA groups: $F_{1,24}=17.21$, $p<0.001$). However, when comparing the groups' post-injection levels expressed as a percentage of baseline the sham or 6-OHDA groups did not differ (Figure 5.5F; Lesion: $F_{1,24}=1.79$, n.s.; Side x Lesion: $F_{1,24}=2.49$, n.s.). Hence, while the 6-OHDA group had lower dopamine baseline levels than sham controls, there was no difference between the percentage change in dopamine levels observed in the sham and 6-OHDA groups following L-DOPA injection.

Food restriction did not affect dopamine levels when including all time points in the analysis (Figure 5.5A-B, D-E; Side x Food restriction: $F_{1,24}=1.79$, n.s.; Food restriction: $F_{1,24}=1.62$, n.s.). Neither was there a difference between the food restricted and *ad libitum* groups when measuring the average post-injection dopamine levels expressed as a percentage of baseline (Figure 5.5C,F; Food restriction: $F_{1,24}=0.30$, n.s.; Side x Food restriction: $F_{1,24}=0.03$, n.s.)

Intra-striatal lesion. The intra-striatal groups exhibited fluctuations in their extracellular dopamine levels over the course of testing (Figure 5.5G-H, J-K; Time: $F_{17,340}=2.32$, $p<0.01$). However, there was no peak or clear time-dependent increase in dopamine levels post L-DOPA injection and the post-injection dopamine levels averaged around 100% of baseline levels (Figure 5.5I,L).

As expected, the dopamine asymmetry in the 6-OHDA groups that had been observed at baseline persisted throughout testing, with lower dopamine levels observed in the lesion relative to intact striata (Figure 5.5J-K; Side x Lesion; $F_{1,20}=10.57$, $p<0.01$; Simple Effect of Side: $\min.F_{1,20}=24.63$, $p<0.001$). Analysis of the average post-injection dopamine levels, expressed as a percentage of baseline, showed increased dopamine levels exclusively in the lesion striatum of the 6-OHDA group (Figure 5.5L; Side x Lesion: $F_{1,20}=4.61$, $p<0.05$; Simple Effect of Lesion: $F_{1,20}=4.77$, $p<0.05$). This suggests that while the intra-striatal 6-OHDA lesion decreased ipsilateral dopamine levels, the lesion striatum was also the only one in which dopamine levels increased post-injection.

Inspection of the data when including all time points in the analysis showed a trend towards reduced dopamine levels in the food restricted groups (Figure 5.5G-H, J-K; Food restriction: $F_{1,20}=3.81$, $p=0.065$). However, there was no effect of food

restriction on the percentage change in dopamine levels post-injection (Food restriction: $F_{1,20}=0.22$, n.s.; Side x Food restriction: $F_{1,20}=0.00$; n.s.). This suggests that while there was a trend towards lower baseline dopamine levels in the food restricted groups, food restriction did not affect the percentage increase in dopamine occurring following acute L-DOPA injection.

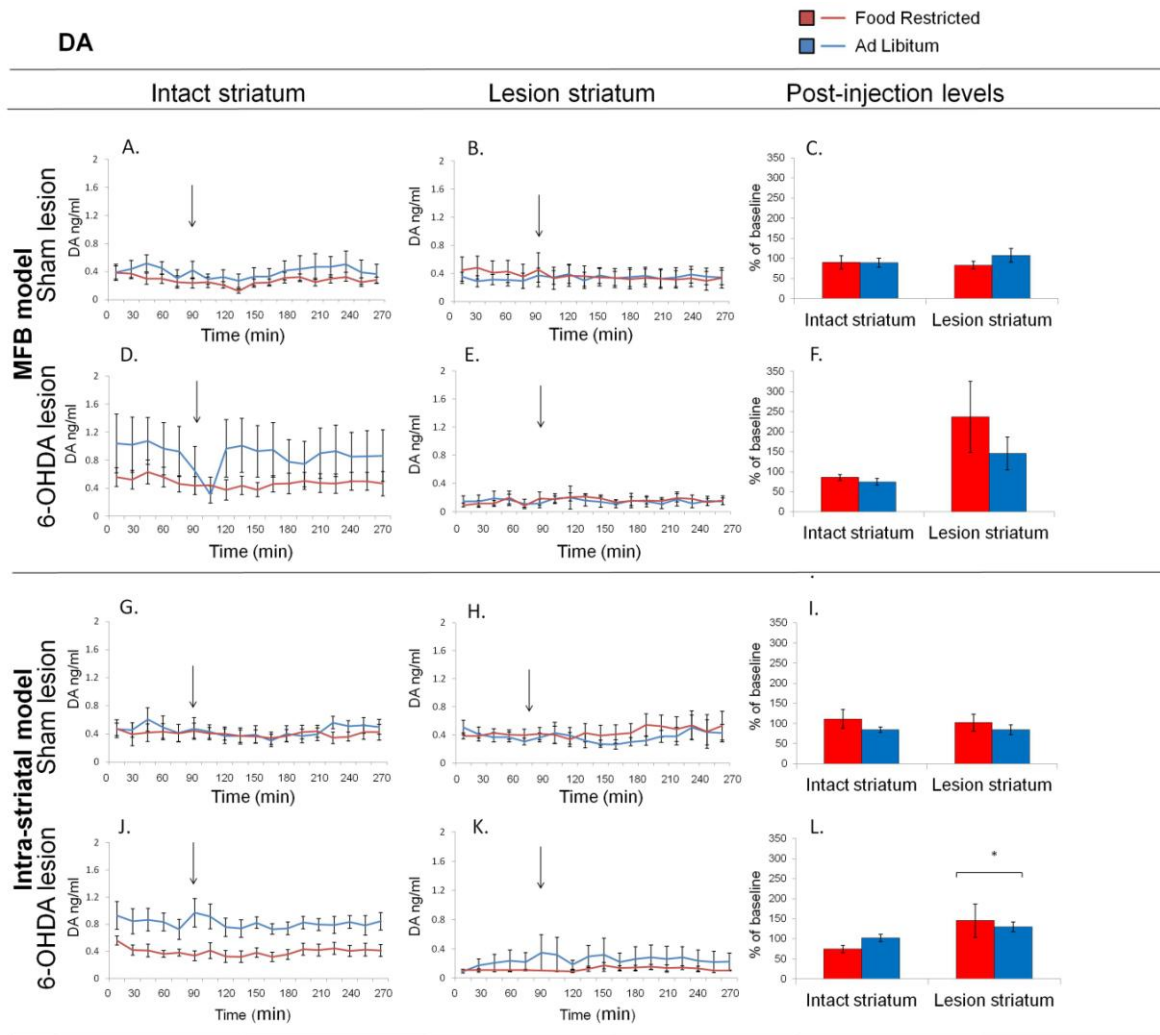


Figure 5.5. The dopamine levels in the intact and lesion striata of rats with sham or 6-OHDA lesions to the MFB (A-F) or the striatum (G-L) that experienced free or restricted access to food. The arrow represents the time point at which rats were administered 5 mg/kg L-DOPA. The column furthest to the right shows the mean post-injection dopamine levels relative to baseline, with 100% corresponding to baseline levels. The error bars show the standard error of the mean. DA=dopamine, MFB=medial forebrain bundle. *Significant difference between sham and 6-OHDA lesion rats. * $p<0.05$

5.3.5. Effect of acute L-DOPA on DOPAC levels

MFB model. Overall, DOPAC levels were lower in the 6-OHDA than in the sham lesion rats (Figure 5.6A-B, D-E; Lesion: $F_{1,24}=5.62$, $p<0.05$). While there were fluctuations in all MFB groups' DOPAC levels there was no observable DOPAC peak following L-DOPA injection (Time: $F_{17,408}=18.33$, $p<0.001$; Time x Lesion: $F_{17,408}=1.16$, n.s.).

Analysis of average post-injection DOPAC levels, expressed as a percentage of baseline showed a higher increase in DOPAC levels in the lesion striatum of 6-OHDA than sham lesion rats (Figure 5.6C,F; Side x Lesion: $F_{1,24}=6.86$, $p<0.05$; Simple Effect of Lesion on Ipsilateral side: $F_{1,24}=6.87$, $p<0.05$). Hence, while 6-OHDA lesions decreased DOPAC levels, acute L-DOPA administration induced a proportionally larger increase in the DOPAC levels in the lesion striata of 6-OHDA lesion than of the sham lesion group.

There was no difference in the DOPAC levels of food restricted and *ad libitum* rats when including all time points in the analysis (Figure 5.6A-B; D-E; Food restriction: $F_{1,24}=0.00$, n.s.; Time x Food restriction: $F_{17,408}=1.10$, n.s.). Neither did food restriction increase the average percentage increase in extracellular DOPA observed following L-DOPA injection (Food restriction: $F_{1,24}=1.39$, n.s.; Side x Food restriction: $F_{1,24}=1.57$, n.s.).

Intra-striatal model. The 6-OHDA lesion rats had overall lower DOPAC levels than sham controls (Figure 5.6G-H, J-K; Lesion: $F_{1,20}=6.43$, $p<0.05$). While all intra-striatal groups showed fluctuations in their DOPAC levels (Time: $F_{17,340}=12.25$, $p<0.001$; Time x Lesion: $F_{17,340}=0.75$, n.s.) there was no clear peak in DOPAC levels post L-DOPA injection.

Analysis of the average DOPAC levels post-injection, expressed as a percentage of baseline, showed an absolute increase in all intra-striatal lesion groups (Figure 5.6I,L). This increase was higher in the lesion striata of the 6-OHDA group than in other groups (Side x Lesion: $F_{1,20}=16.15$, $p<0.01$; Simple Effect of Lesion: $F_{1,20}=16.66$, $p<0.01$).

Food restriction did not affect DOPAC levels when including all time points in the analysis (Figure 5.6G-H, J-K; Food restriction: $F_{1,20}=0.09$, n.s.; Time x Food restriction: $F_{17,340}=1.23$, n.s.). Neither did it impact on the percentage change in

DOPAC levels following L-DOPA injection (Figure 5.6I, L; Food restriction: $F_{1,20}=1.71$, n.s.; Side x Food restriction: $F_{1,20}=1.41$, n.s.).

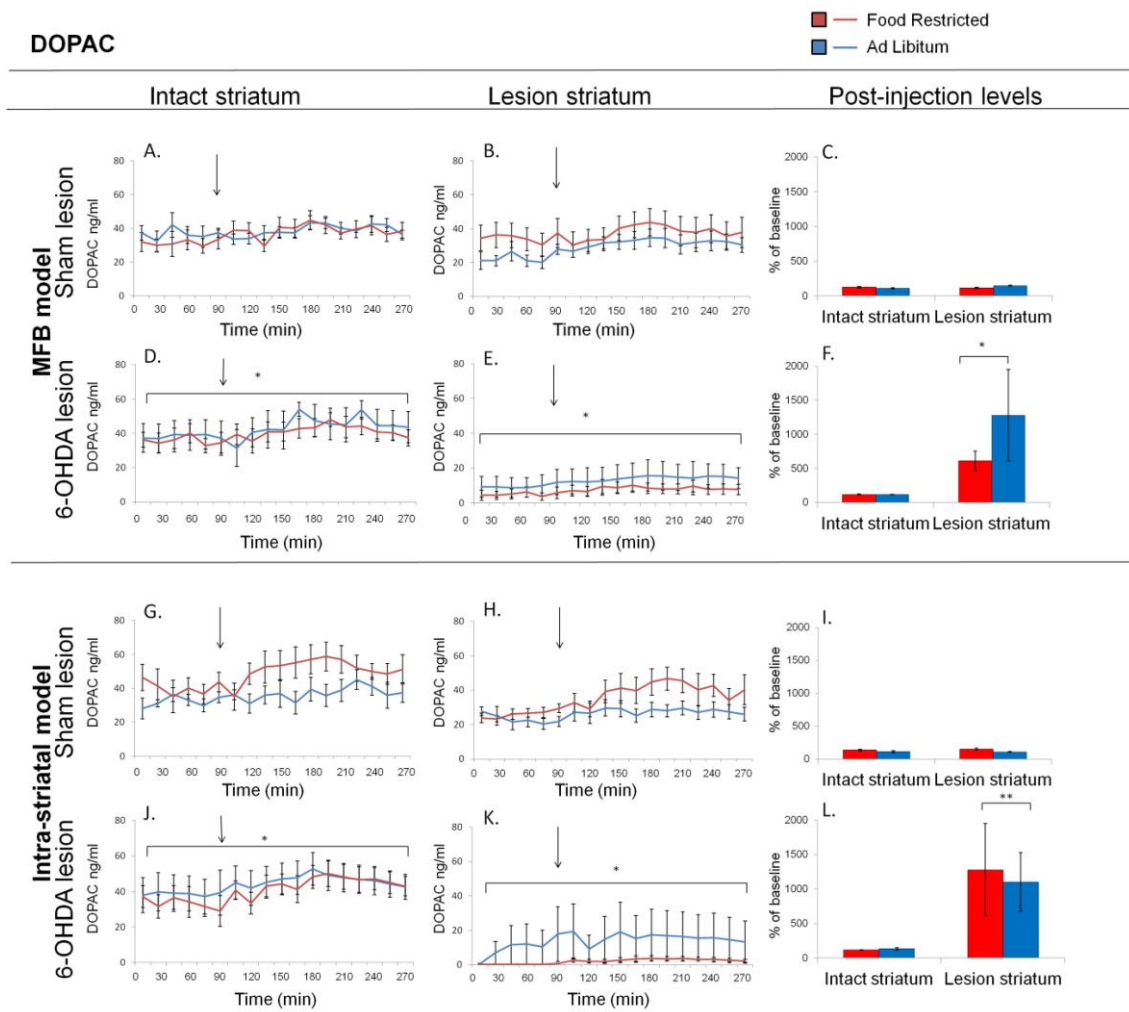


Figure 5.6. The DOPAC levels in the intact and lesion striata of rats with sham or 6-OHDA lesions to the MFB (A-F) or the striatum (G-L) that experienced free or restricted access to food. The arrow represents the time point at which rats were administered 5 mg/kg L-DOPA. The column furthest to the right shows the mean post-injection DOPAC levels relative to baseline, with 100% corresponding to baseline levels. The error bars show the standard error of the mean. MFB=Medial forebrain bundle. *Significant difference between sham and 6-OHDA lesion rats. * $p<0.05$, ** $p<0.01$

5.3.6. Effect of acute L-DOPA on HVA levels

MFB model. While there was no peak in HVA levels post L-DOPA injection, there was a slight increase in the extracellular HVA levels in the 6-OHDA group's intact striata (Figure 5.7A-B, D-E; Side x Time x Lesion: $F_{17,408}=2.63$, $p<0.001$; Simple Effect of Time: $F_{8,17}=3.74$, $p<0.05$). Average post-injection HVA levels, expressed as a percentage of baseline, confirmed a greater increase in the HVA levels of the 6-OHDA lesion groups' lesion striatum relative to their intact striatum and the sham lesion group (Figure 5.7C,F; Side x Lesion: $F_{1,24}=6.71$, $p<0.05$; Simple Effect of Side: $F_{1,24}=16.45$, $p<0.001$). This suggests that while 6-OHDA MFB lesions decrease HVA levels, which are indicative of dopamine turnover, acute L-DOPA administration preferentially increase dopamine turnover in the lesion striata.

Intra-striatal model. Whilst there was no clear HVA peak post-injection, there was a gradual increase in extracellular HVA following acute L-DOPA administration (Figure 5.7G-H, J-K; Time: $F_{17,340}=18.42$, $p<0.001$). The change in HVA levels was similar between food restricted and *ad libitum* rats (Time x Food restriction: $F_{17,340}=0.65$, n.s.). While both 6-OHDA and sham lesion rats showed a slight increase in their HVA levels over the course of testing, the 6-OHDA lesion rats consistently had lower absolute HVA levels in their lesion striata than sham controls (Side x Time x Lesion: $F_{17,340}=1.81$, $p<0.05$; Simple Effect of Lesion: $F_{1,20}=6.39$, $p<0.05$). Nonetheless, measuring the average HVA levels expressed as a percentage of baseline showed that the relative increase in HVA levels was greater in the 6-OHDA than in the sham lesion groups (Figure 5.7I,L; Lesion: $F_{1,20}=8.34$, $p<0.05$). Visual inspection of the data suggested this was driven by greater percentage increase specifically in the lesion striata of 6-OHDA rats, and the statistical analysis showed a strong trend towards supporting this (Side x Lesion: $F_{1,20}=4.10$, $p=0.056$).

Food restriction decreased HVA levels in the 6-OHDA rats but not in intact controls (Figure 5.7G-H, J-K; Food restriction x Lesion: $F_{1,20}=4.51$, $p<0.05$; Simple Effect of Food restriction: $F_{1,20}=5.77$, $p<0.05$). However, despite differences in the baseline samples, food restricted and *ad libitum* groups did not differ in terms of overall percentage HVA increase observed following L-DOPA administration (Figure 5.7I,L; Food restriction: $F_{1,20}=1.48$, n.s.; Side x Food restriction: $F_{1,20}=0.34$, n.s.)

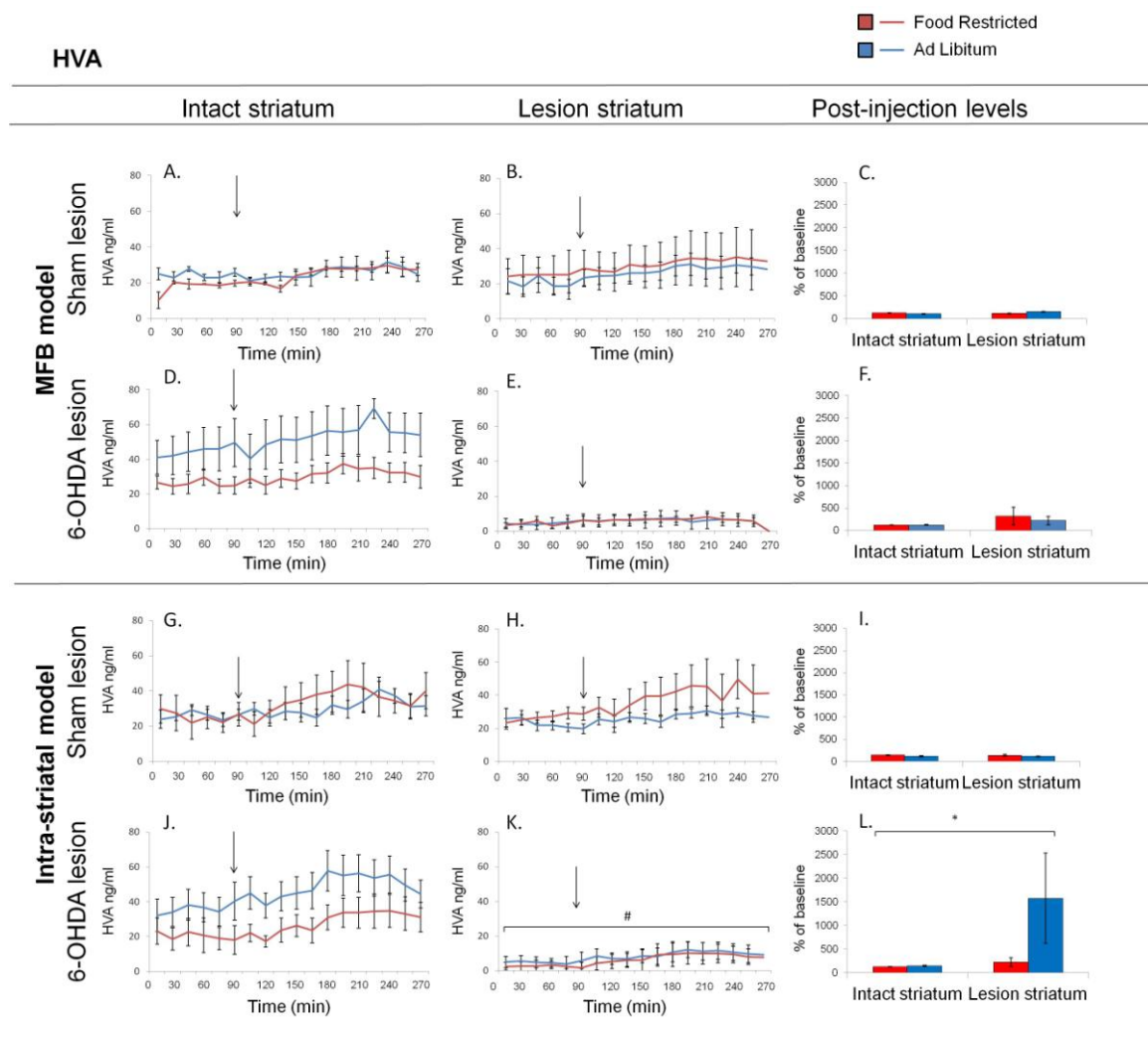


Figure 5.7. The HVA levels in the intact and lesion striata of rats with sham or 6-OHDA lesions to the MFB (A-F) or the striatum (G-L) that experienced free or restricted access to food. The arrow represents the time point at which rats were administered 5 mg/kg L-DOPA. The column furthest to the right shows the mean post-injection HVA levels relative to baseline, with 100% corresponding to baseline levels. The error bars show the standard error of the mean. \diamond Significant difference between the 6-OHDA lesion group's ipsilateral striatum, relative to their contralateral striatum and either of the sham lesion group's striata, *Significant difference between sham and 6-OHDA lesion rats. # $p < 0.05$, * $p < 0.05$, MFB=Medial forebrain bundle.

5.3.7. Effect of acute L-DOPA on DOPAC / DA ratio

MFB model. Dopamine utilization was marginally higher in the sham than the lesion group when including all time points in the analysis (Figure 5.8A-B, D-E; Lesion:

$F_{1,24}=5.27$, $p<0.05$; Side x Lesion: $F_{1,24}=0.02$, n.s.). In addition, the sham lesion group's dopamine utilization fluctuated more over the course of testing, causing the sham group to have significantly higher ratio than the lesion group both before (60-90 min into testing) and after (105, 135, 150, 180, and 210 min into testing) acute L-DOPA administration (Time x Lesion: $F_{17,408}=2.18$, $p<0.01$; Simple Effect: $F_{1,24}=4.42$, $p<0.05$). There was no effect of food restriction on DOPAC/DA ratio (Food restriction: $F_{1,25}=0.04$, n.s.; Side x Food: $F_{1,25}=1.88$, n.s.).

The percentage change in dopamine utilization after acute L-DOPA administration did not differ between the 6-OHDA and sham lesion groups (Figure 5.8C,F; Lesion: $F_{1,24}=1.14$, n.s.), between the lesion and intact striata (Side: $F_{1,24}=1.75$, n.s.; Side x Lesion: $F_{1,24}=0.01$, n.s.) or between *ad libitum* and food restricted rats (Food restriction: $F_{1,24}=0.18$, n.s.; Side x Food restriction: $F_{1,24}=0.04$, n.s.).

Intra-striatal model. Overall, dopamine utilization was slightly higher in the sham than in the 6-OHDA lesion rats (Figure 5.8G-H, J-K; Lesion: $F_{1,20}=24.52$, $p<0.001$), and in food restricted versus *ad libitum* rats (Food restriction: $F_{1,20}=10.01$, $p<0.01$). There was also a trend towards higher dopamine utilization in the intact than lesion striata (Side: $F_{1,20}=3.74$, $p=0.067$; Side x Lesion: $F_{1,20}=1.60$, n.s.; Side x Food restriction: $F_{1,21}=0.84$, n.s.).

There were significant fluctuations in the dopamine utilization measured in the sham but not 6-OHDA lesion group (Time: $F_{17,340}=7.10$, $p<0.001$; Time x Lesion: $F_{17,340}=1.86$, $p<0.05$; Simple Effect: $F_{4,17}=10.40$, $p<0.05$) which inspection of the data suggested was due to outliers in the sham lesion group skewing the data. Interestingly, the analysis also showed a significant difference between the change in dopamine utilization of the *ad libitum* and food restricted rats, with the latter showing increased dopamine utilization over the course of testing (Time x Food restriction: $F_{17,340}=1.72$, $p<0.05$; Simple Effect: $F_{4,17}=8.56$, $p<0.05$).

Analysis of the percentage change in dopamine utilization after acute L-DOPA injection showed that, overall, the 6-OHDA lesion rats' dopamine utilization increased less than that of sham controls (Figure 5.8I,L; Lesion: $F_{1,20}=12.08$, $p<0.01$; Side x Lesion: $F_{1,20}=2.93$, n.s.). Interestingly, there was also an effect of food restriction, which was associated with an overall greater increase in dopamine utilization relative to *ad libitum* controls (Food restriction: $F_{1,20}=10.00$, $p<0.01$). While this difference was numerically higher in the 6-OHDA lesion group's lesion

than intact striata, the side specific increase did not meet statistical significance (Side x Food restriction: $F_{1,20}=0.09$, n.s; Side x Lesion x Food restriction: $F_{1,20}=0.85$, n.s.).

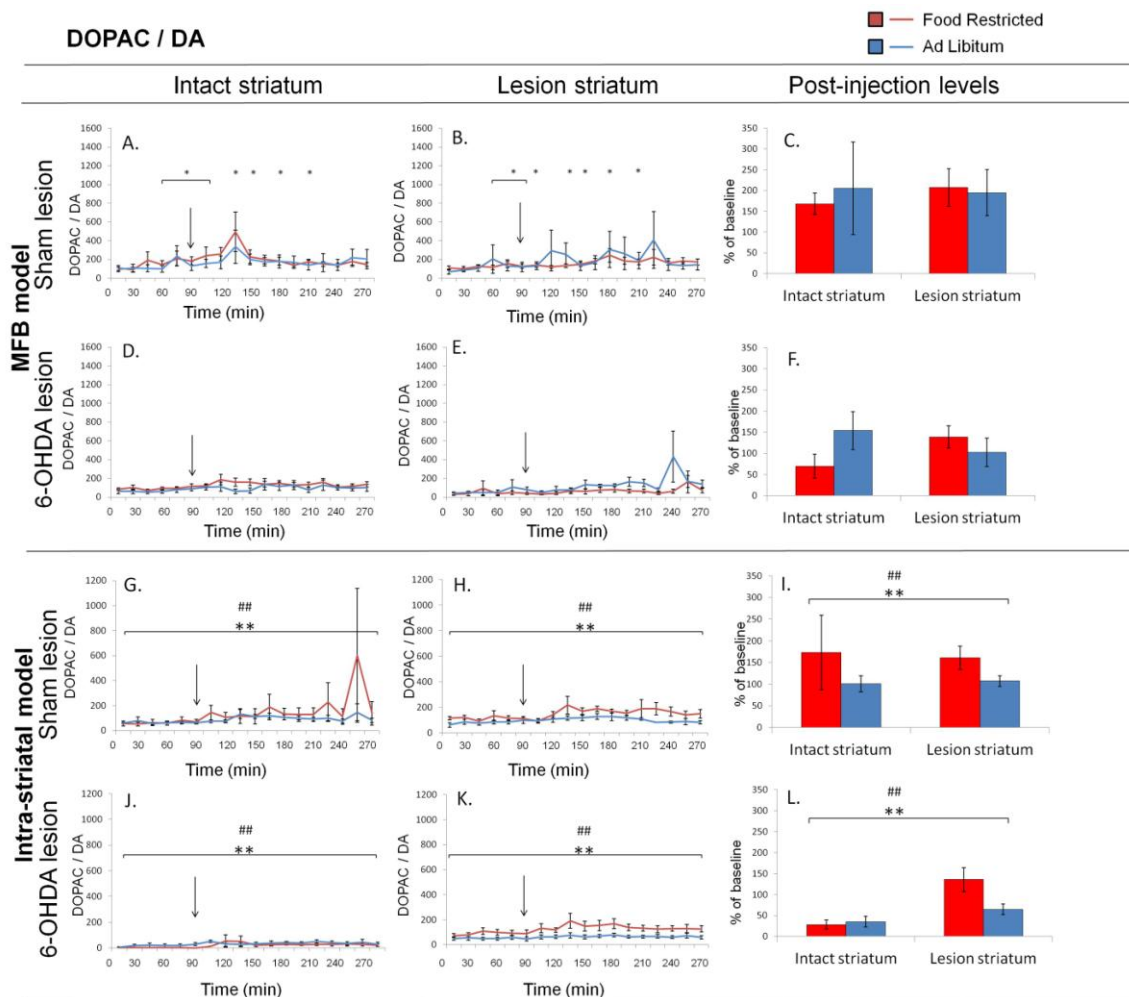


Figure 5.8. The DOPAC/DA ratio in the intact and lesion striata of rats with sham or 6-OHDA lesions to the MFB (A-F) or the striatum (D-L) that experienced free or restricted access to food. The arrow represents the time point at which rats were administered 5 mg/kg L-DOPA. The column furthest to the right shows the mean post-injection 5HIAA levels relative to baseline, with 100% corresponding to baseline levels. The error bars show the standard error of the mean. MFB=Medial forebrain bundle. #Significant difference between *ad libitum* and food restricted rats, *Significant difference between sham and 6-OHDA lesion rats. ## $p<0.01$, ** $p<0.01$.

5.3.8. Effect of acute L-DOPA on 5HIAA levels

MFB model. While extracellular 5HIAA levels fluctuated throughout the testing session there was no peak, or clear time-dependent increase, following L-DOPA injection (Figure 5.9A-B, D-E; Time: $F_{17,408}=1.97$, $p<0.05$; Time x Lesion:

$F_{17,408}=1.11$, n.s.). Measuring the post-injection 5HIAA levels as a percentage of baseline confirmed that there was little change in extracellular 5HIAA levels after acute L-DOPA, with the exception of the 6-OHDA lesion *ad libitum* group, where one outlier increased the variation in the data (Figure 5.9C,F; Side x Lesion: $F_{1,24}=2.87$, $p<0.05$; Effect of Side in 6-OHDA lesion group: $F_{1,24}=5.62$, $p<0.05$)

In the sham lesion groups, food restriction was associated with higher 5HIAA levels relative to *ad libitum* controls. In the 6-OHDA lesion group, the reverse trend was observed in the intact striata (Side x Lesion x Food restriction: $F_{1,24}=7.28$, $p<0.05$; Simple Effect of Food restriction: $\min.F_{1,24}=5.03$, $p<0.05$). However, analysing post-injection 5HIAA levels expressed as a percentage of baseline did not show a difference between *ad libitum* and food restricted rats (Food restriction: $F_{1,24}=2.23$, n.s.; Side x Food restriction: $F_{1,24}=2.69$, n.s.). This suggests that while there were baseline differences between the *ad libitum* and food restricted groups they did not differ in the relative change of their 5HIAA levels post L-DOPA injection.

Intra-striatal model. While the intra-striatal cohort showed fluctuations in their extracellular 5HIAA levels, which were especially pronounced in the lesion striatum (Figure 5.9G-H, J-K; Side x Time: $F_{17,340}=1.66$, $p<0.05$; Effect of Time on lesion side: $F_{4,17}=8.51$, $p<0.05$), there was no peak in 5HIAA levels post L-DOPA injection. While there was a significant difference in the percentage increase in 5HIAA levels observed following acute L-DOPA in the 6-OHDA and sham lesion groups, inspection of the raw data suggested this was due to an outlier in the 6-OHDA food restricted group (Figure 5.8I,L; Lesion: $F_{1,20}=9.57$, $p<0.05$; Side x Lesion: $F_{1,20}=0.03$, n.s.).

There was no overall effect of food restriction on 5HIAA levels when including all time points in the analysis (Figure 5.9G-H, J-K; Food restriction: $F_{1,20}=2.24$, n.s.). However, visual inspection of the data suggested lower 5HIAA levels specifically in the intact striatum of the 6-OHDA lesion group and there was a strong trend supporting this (Side x Lesion x Food restriction: $F_{1,20}=4.32$, $p=0.05$). There was no significant difference in the percentage increase in 5HIAA levels following acute L-DOPA injection (Figure 5.8I, L; Food restriction: $F_{1,20}=1.79$, n.s.; Side x Food restriction: $F_{1,20}=0.94$, n.s.)

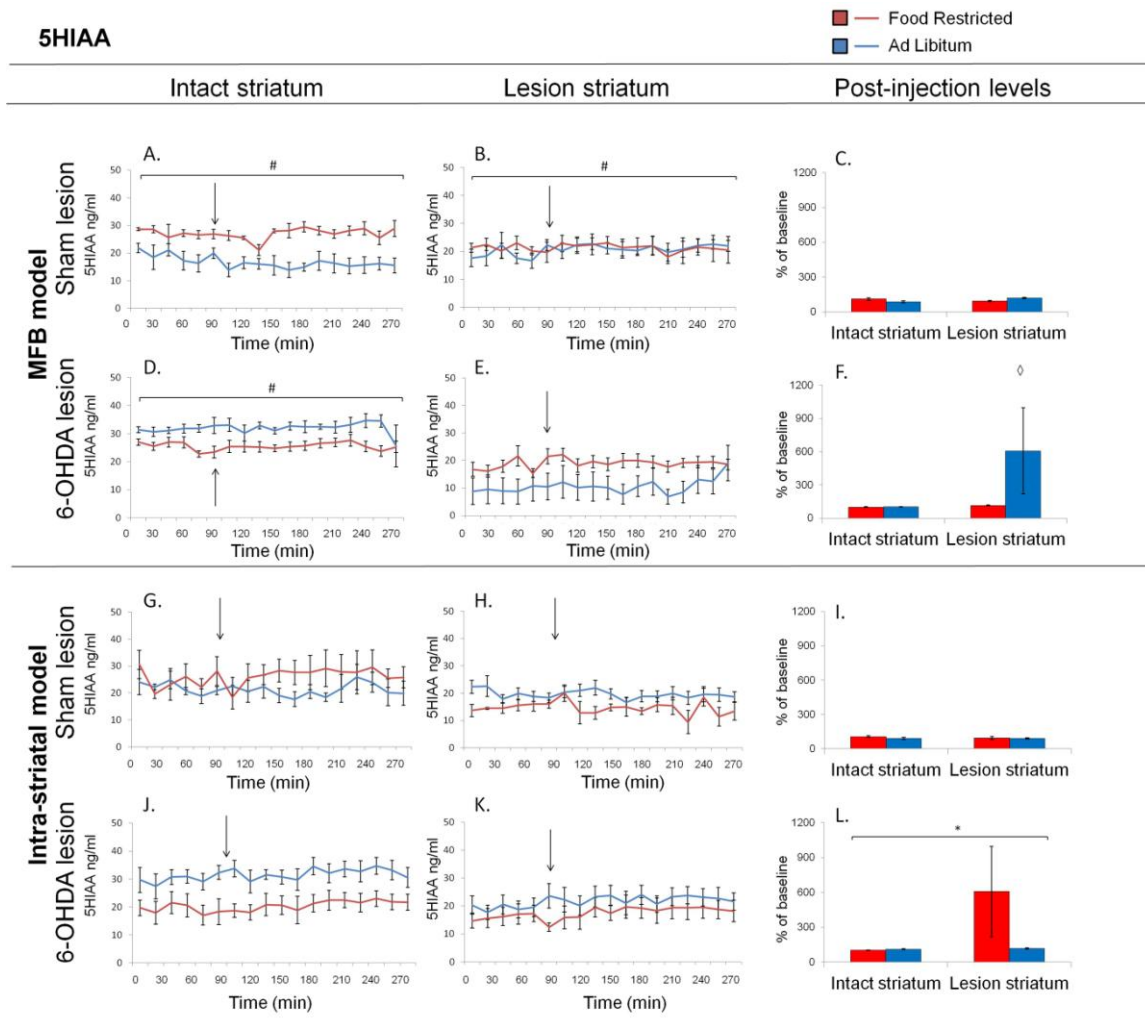


Figure 5.9. The 5HIAA levels in the intact and lesion striata of rats with sham or 6-OHDA lesions to the MFB (A-F) or the striatum (D-L) that experienced free or restricted access to food. The arrow represents the time point at which rats were administered 5 mg/kg L-DOPA. The column furthest to the right shows the mean post-injection 5HIAA levels relative to baseline, with 100% corresponding to baseline levels. The error bars show the standard error of the mean. MFB=Medial forebrain bundle. *Significant difference between sham and 6-OHDA lesion rats, #Significant difference between ad libitum and food restricted rats * $p < 0.05$, # $p < 0.05$

5.4. Discussion

Lesion rats with free or restricted access to food were included in a microdialysis study to test the hypothesis that food restriction increased the amount of L-DOPA that crosses the BBB. The hypothesis was tested by comparing extracellular DOPA levels following acute L-DOPA administration in rats with free or restricted access to food.

DOPA is the precursor to dopamine and converted into the latter by the enzyme DOPA decarboxylase (Flatmark *et al* (2000)). To provide information on lesion success and the ability for the administered dose of acute L-DOPA to increase dopamine levels and dopamine utilization, which are indicative of the therapeutic effect L-DOPA exerts, the experiment also measured dopamine, its metabolites DOPAC and HVA, and the DOPAC/dopamine ratio. In line with previous experiments (e.g. Lindgren *et al*, 2010), the success of the unilateral 6-OHDA lesions was demonstrated by a pronounced asymmetry in the dopamine levels of lesion rats' intact and lesion striata. Interestingly, the lesion did not only decrease ipsilateral dopamine levels but also increased extracellular dopamine in the intact striata. Previous studies have demonstrated increased TH proteins levels in the intact striatum following unilateral 6-OHDA lesions to the striatum (Kozlowski *et al*, 2004). In addition, unilateral nigral 6-OHDA lesions have been shown to increase both dopamine levels in contralateral striatal tissue and levels of extracellular dopamine in the contralateral striatum (Zhang *et al*, 1988). These data are consistent with the current findings and have previously been hypothesised to represent a compensatory mechanism (Kozlowski *et al*, 2004), which has been suggested to be mediated by increased dopamine release per terminal or an increase in the number of dopamine terminals in the contralateral hemisphere (Zhang *et al*, 1988).

5.4.1. Effect of food restriction on L-DOPA influx

The aim of the current experiment was to directly test the hypothesis that food restriction increases L-DOPA influx via the BBB. The hypothesis was tested in the intra-striatal and MFB 6-OHDA models of PD by measuring striatal extracellular levels of DOPA, dopamine, and dopamine metabolites in freely moving rats before and after acute L-DOPA administration. The data did not show a difference in the extracellular DOPA levels of food restricted and *ad libitum* rats, and as such did not support the hypothesis.

Previous studies have reported a peak in MFB lesion rats' extracellular DOPA and dopamine levels approximately 40 min after acute administration of 6 mg/kg L-DOPA (Carta *et al*, 2006; Lindgren *et al*, 2010). A similar DOPA peak was observed in the current experiment following 5 mg/kg L-DOPA (Figure 5.4). However, there was no peak in the extracellular dopamine levels observed after L-DOPA injection. Whilst the absence of a dopamine peak was unexpected, other studies have failed to find a noticeable peak in extracellular dopamine levels of intact L-DOPA naïve rats unless using a L-DOPA dose ≥ 25 mg/kg, although it should be noted that the authors did not report the accompanying

benserazide dose (Abercrombie *et al*, 1990). Other microdialysis studies, including one utilizing drug naïve MFB lesion rats report a dopamine peak 1 h after acute administration when 15 mg/kg L-DOPA + 10 mg/kg benserazide were administered (Jonkers *et al*, 2000). Hence, previously published literature show differences not only between the doses required to measure a peak in extracellular dopamine in intact versus lesion rats, but also in the time post-injection when the peak is observed. It is possible that a higher L-DOPA dose, such as the 15 mg/kg used by Jonkers and colleagues (2000), would have been required for an extracellular dopamine peak to be observed in the current experiment. A higher dose was not used because a pilot study (data not reported) in which 10 mg/kg L-DOPA was administered to MFB lesion rats during microdialysis testing demonstrated AIMs of sufficient magnitude for rats to risk entangling themselves in the tubing as well as L-DOPA induced rotations of sufficient magnitude to twist the tubing and thereby interrupting aCSF flow. Therefore, for animal welfare reasons as well as to minimise the number of samples that may have been lost due to disrupted aCSF flow, the lower dose of 5 mg/kg L-DOPA was used. The choice of dose was justified based on (i) previous dose response curves (Chapter 4) demonstrating an effect of 5 mg/kg L-DOPA on motor function in lesion rats, and (ii) the observation that the AIMs and L-DOPA induced rotations observed in L-DOPA naïve MFB lesion rats at this dose did not disrupt microdialysis testing.

The food restricted groups did not show significantly higher DOPA levels than *ad libitum* controls and the current data therefore suggest that, contrary to the hypothesis, food restriction does not increase L-DOPA influx in the rat striatum. There are, however, caveats associated with the use of microdialysis which may have influenced the measured DOPA levels. For example, it is possible that the probe implantation 48 h prior to surgery disrupted the blood brain barrier, thereby decreasing the likelihood of observing a difference between the *ad libitum* and food restricted groups. Previously published studies measuring BBB permeability have shown that microdialysis probe implantation causes an acute increase in the permeability of the BBB of male Sprague Dawley rats 1-2 h after insertion, with the maximum effect on permeability being observed 1-2 days after microdialysis implantation (Groothuis *et al*, 1998). To comply with the project license under which the current experiment was conducted, the microdialysis session was conducted 48 h after microdialysis implantation. Previous in-house studies (not published) using the same interval between probe implantation and microdialysis have successfully measured increases in DOPA levels after acute L-DOPA injection using the same set-up and it was therefore not expected that increased BBB permeability would interfere with the experiment. However, considering the

findings by Groothuis and colleagues (1998), the possibility that an increased permeability in the BBB at the time of testing led to an overall increase in L-DOPA influx, thereby reducing the likelihood of observing a difference between the food restricted and *ad libitum* rats cannot be excluded.

5.4.2. Effect of food restriction on dopamine levels and utilization

While the aim of the experiment was to test the effect of food restriction on L-DOPA influx it also reported levels of dopamine and its metabolites as well as the DOPAC/dopamine ratio, which has previously been used to estimate dopamine utilization (e.g. Cartmell *et al*, 2000; Jentsch *et al*, 1998). Including these measurements in the analysis supplemented data on DOPA influx by providing insight into not only extracellular dopamine levels but also the control and experimental groups' utilization of the available dopamine.

Restricting rats' food intake in the current experiment produced a strong trend towards reduced baseline dopamine levels in the intra-striatal model (Figure 5.3D; $p=0.058$) accompanied by increased dopamine utilization (Figure 5.3J, 5.8J). Previous publications have demonstrated that acute food restriction decrease dopamine clearance rates in intact rats (Sevak *et al*, 2008), and that feeding young (20-25 g) rats a low-protein diet for 1 year reduces striatal dopamine levels (Mathangi & Namasivayam, 2001). In addition, 72 hours food restriction has been shown to increase striatal DOPAC/dopamine ratio in rats (Tsuji *et al*, 1988). The current findings of decreased dopamine levels and increased dopamine utilization in food restricted rats with intra-striatal lesions thus align with existing literature on the effect of food restriction on intact rats. While the reason for these changes was not directly tested it is noted that L-tyrosine (the precursor for DOPA) is derived from food and that L-phenylalanine (the precursor for L-tyrosine) is either derived from food or synthesised from L-tyrosine (Figure 1.5; Flatmark *et al*, 2000). Changes in diet or quantity of food intake may thus be hypothesised to indirectly affect dopamine levels by affecting transmitters further down its synthesis pathway. This would be in line with previous data demonstrating an effect of diet on brain tyrosine levels (Wurtman *et al*, 1980). The increased dopamine utilization observed following food restriction in the intra-striatal lesions may in turn represent a compensatory mechanism. It has previously been demonstrated that the dopaminergic system is able to compensate for dopamine loss by increasing the dopamine utilization in 6-OHDA lesion rats (Hefti *et al*, 1985) and MPTP lesion monkeys (Pifl *et al*, 2006), a phenomenon which has been hypothesised to reflect a compensatory mechanism (Hornykiewicz, 1998). It is thus plausible that decreased dopamine levels caused by dietary

changes may induce a similar compensatory response, which could explain the increased DOPAC/dopamine ratio observed in the food restricted rats with intra-striatal lesions.

The rotational bias exhibited by rats with unilateral 6-OHDA lesions is believed to reflect the dopamine asymmetry between the lesion and intact striata in rats with unilateral lesions (Ungerstedt, 1968, 1970). If food restriction decreases dopamine levels in the intact but not lesion striatum, it follows that food restriction would indirectly decrease the dopamine asymmetry between the two hemispheres. It may be therefore be hypothesised that, because of this decreased asymmetry, less L-DOPA was required to restore the dopamine balance between the intact striata and lesion striata in food restricted relative to *ad libitum* lesion rats. If true, it would also follow that less L-DOPA would be required to shift the dopamine balance from the intact to the lesion striata in food restricted relative to *ad libitum* rats and to produce a contralateral rotational bias. While an untested hypothesis, it may explain why the dose response curves in Chapter 4 showed greater rotational bias in food restricted than *ad libitum* intra-striatal lesion rats despite the current chapter not demonstrating a greater L-DOPA influx in food restricted rats.

5.4.3. Effect of food restriction on the serotonergic system

In addition to DOPA, dopamine, and dopamine metabolites the current experiment also measured levels of the serotonin metabolite 5HIAA. Serotonin is formed from L-tryptophan which is supplied to the central nervous system via the blood, and levels of which are affected by diet (Fernstrom, 1981). Following MAO initiated catabolism of serotonin, the neurotransmitter is broken down to 5HIAA (Fernstrom, 1981) which was included as a control measurement in the current microdialysis experiment. Following acute L-DOPA injection, there was a clear peak in extracellular DOPA but not 5HIAA levels which suggested that the injection induced changes observed in the microdialysis data were specific to DOPA and the dopaminergic system.

In addition to decreased dopamine levels, 6-OHDA lesions to the MFB or striatum also decreased baseline 5HIAA levels. This effect of dopamine depletion on 5HIAA levels is in accordance with previously published data showing reduced striatal 5HIAA levels as well as decreased firing in dorsal raphé serotonergic neurons following dopamine depletion (Karstaedt *et al*, 1994; Guiard *et al*, 2008).

Furthermore, the data demonstrated altered 5HIAA levels following food restriction. Both the MFB and intra-striatal 6-OHDA models showed decreased 5HIAA levels in their intact striata following food restriction. These findings are in line with previous publications

showing decreased serotonin and 5HIAA levels after food restriction of intact rats (Schweiger *et al.*, 1989; Mathangi & Namasivayam, 2001). Interestingly, food restriction had different effects on the 5HIAA levels in the *lesion* striatum of rats with MFB or intra-striatal 6-OHDA lesions. While rats with MFB lesions showed increased ipsilateral 5HIAA levels following food restriction, no effect was observed in the intra-striatal cohort. The two lesion models differ in the extent of the nigral dopamine loss they produce, with more pronounced loss being observed following MFB than intra-striatal lesions (e.g. Yuan *et al.*, 2005). Previous studies have demonstrated increased striatal levels of serotonin and 5HIAA following >90% loss of nigral dopamine in 6-OHDA lesion rats (Zhou *et al.*, 1991) which is similar to the nigral loss induced by MFB lesions. It is therefore possible that the observed differences between ipsilateral 5HIAA levels in the MFB and intra-striatal model were linked to differences in the striatal serotonergic sprouting they produce, as serotonergic sprouting only occurs following near complete dopamine depletion (Zhou *et al.*, 1991).

5.4.4. Conclusions

The aim of the experiment was to test the hypothesis that food restriction increases L-DOPA influx in the rat. 6-OHDA lesions to the MFB and striatum decreased dopamine levels and dopamine utilization (as measured by DOPAC/DA levels and HVA) in the lesion striatum. Injection of acute L-DOPA produced a measurable peak in extracellular DOPA levels but there was no effect of food restriction, with similar extracellular DOPA levels observed in both food restricted and *ad libitum* rats. Hence, the data did not support the hypothesis that food restriction increases L-DOPA influx to the striatum. Based on the current data it cannot, as previously hypothesised, be concluded that the increased rotational bias in food restricted relative to *ad libitum* intra-striatal lesion rats observed in Chapter 4 was due to higher L-DOPA influx.

6. Effect of Dopaminergic Treatment on the Acquisition and Maintenance of Motor Skills

Background: Current literature suggests that synaptic plasticity may enhance or mediate learning of new motor skills, while not being essential for the maintenance of already acquired skills. The onset of LID has been shown to disrupt synaptic plasticity in the rat MFB model of PD. Based on previously published literature, it was therefore hypothesised that LID onset would impair acquisition of a novel motor skill, but not performance of a skill that had been acquired prior to lesion and chronic L-DOPA treatment.

Aim: The aim of the chapter was to test whether chronic L-DOPA administration, which induces LID in the vast majority of MFB lesion rats would (i) impair the rate with which MFB lesion rats learn a motor task *de novo*, and (ii) affect performance on the same task in lesion rats that had learned the task prior to lesion and L-DOPA treatment.

Methods: The hypothesis was tested by measuring performance on the staircase task. Experiment 6 compared post-lesion performance of intact, lesion saline treated, and lesion L-DOPA treated rats that had learned the task prior to lesion and drug treatment. Experiment 7 compared the performance of intact, lesion saline treated, and lesion L-DOPA treated rats when having to learn the task *de novo* after lesions. Experiment 8 was designed to replicate the findings from Experiment 7 while also including an intact and lesion group treated with the D2 agonist bromocriptine.

Results: As hypothesised, chronic L-DOPA did not affect performance of rats that had learned the staircase task prior to lesion and L-DOPA treatment. An L-DOPA induced impairment in the acquisition of the task was found in Experiment 7 but not in Experiment 8. The presented data were therefore insufficient to determine whether or not LID onset impairs acquisition of the staircase task.

6.1. Introduction

It has long been known that dopamine is involved in the execution of motor actions. However, another crucial aspect of motor function is the ability to learn novel motor actions and to refine pre-existing motor skills. Motor skill learning is a complex procedure, which is believed to be mediated by the cortico-cerebellar and cortico-striatal pathways. Whilst the cortico-cerebellar pathway is believed to play a role in the spatial awareness that is required for the execution of motor actions, the cortico-striatal pathway is believed to hold representations of the individual motor actions that form part of a motor sequence (Hikosaka *et al*, 2002). Measuring neural activation in healthy participants as they either perform a previously learned motor sequence, or learn a novel motor sequence, has demonstrated that only the latter is associated with significant activation in the prefrontal cortex (Jenkins *et al*, 1994). In addition, there was greater activation in the cerebellum, lateral premotor cortex, and parietal cortex during *de novo* learning. Conversely, the putamen was activated in both *de novo* learning and during performance on a previously learned sequence, suggesting that the basal ganglia are implicated in both acquisition and maintenance of motor skills (Jenkin *et al*, 1994). As predicted from imaging studies of healthy controls, both PD patients and patients with lesions to the cerebellum are impaired relative to healthy in their ability to learn a novel motor sequence (Doyon *et al*, 1994), and PD patients are also been shown to be impaired in their ability to adapt a motor repertoire to changes in the environment (Krebs *et al*, 2011).

In line with clinical data, preclinical findings also implicate the basal ganglia in the acquisition of novel motor skills. Some of these preclinical findings have been accomplished using the skilled reach task, which measures the success with which rats grasp and retrieve food pellets placed on a pedestal located 1.5 cm away from them. Using this paradigm, Luft *et al* (2004) demonstrated impaired acquisition of the paw-reaching skill following infusion of the protein synthesis inhibitor anisomycin, which inhibits HFS induced LTP (Okulski *et al*, 2002), to the motor cortex. Conversely, if anisomycin was infused *after* the task had already been acquired it did not affect performance (Luft *et al*, 2004). The effect of infusing anisomycin into the dorsal striatum, which is connected to the motor cortex, on motor learning, was studied in a separate experiment (Wächter *et al*, 2010). This study utilised a similar apparatus, in which rats could open an automatic door in their test cage by nose poking. Once open, rats could reach for a food pellet located 1.5 cm away using their preferred paw. In line with Luft *et al*'s (2004) data, Wächter and colleagues (2010) found that infusing anisomycin into the dorsal striatum prior to motor task training decreased the

number of successful reaches the rats made. Conversely, if the infusion was made once a plateau in the rats' motor performance had been reached the anisomycin did not affect behaviour. In neither condition did anisomycin affect the time taken for rats to initiate new trials, which was taken to suggest that the impaired motor learning was not mediated by decreased motivation (Wächter *et al*, 2010). The role of synaptic plasticity in motor skill learning was further investigated by Yin *et al* (2009). Using in-vivo and ex-vivo recordings from mice that received rotarod training, Yin and colleagues (2009) showed an increase in neuronal activity and synaptic strength in the dorsomedial striatum during early motor training that was transferred to the dorsolateral striatum once the skill had been fully learned. The data by Yin *et al* (2009) thus demonstrated striatal synaptic plasticity in the dorsomedial striatum during the acquisition of novel motor skills, and in the dorsolateral striatum during the maintenance of recently learned motor skills. While the precise role of synaptic plasticity in motor learning has been debated (e.g. Conner *et al*, 2003; Kilgard, 2012), the studies described above suggest (i) that the cortico-striatal pathway plays a role in the acquisition of motor skills, and (ii) that not only dopamine but also synaptic reorganization is involved in motor skill acquisition.

As previously discussed in section 1.6, the onset of LID is associated with disrupted synaptic plasticity in the dopaminergic system (Picconi *et al*, 2003; Prescott *et al*, 2009; Huang *et al*, 2011). In the rat MFB model, the onset of LID is associated with restored ability for LTP formation but lost ability for depotentiation in the cortico-striatal pathway (Picconi *et al*, 2003). Based on previously published data showing an involvement of striatal synaptic plasticity in motor skill acquisition, it was hypothesised that LID onset in the MFB model would render rats less able to refine their motor repertoire during motor skill learning, and that LID onset would therefore interfere with the acquisition, but not maintenance, of motor skills. Three experiments were conducted to test the hypothesis. Experiment 6 tested the effect of MFB lesions and chronic L-DOPA treatment, which induces LID in the majority of MFB lesion rats (Winkler *et al*, 2002), on pre-trained rats' ability to maintain successful reaching in the staircase boxes. Experiment 7 tested the effect of lesions and chronic L-DOPA treatment on rats' ability to learn to retrieve sucrose pellets from staircase boxes *de novo*. Experiment 8 was conducted to replicate the findings from Experiment 7 whilst also including a lesion and intact group chronically treated with the D2 agonist bromocriptine. Bromocriptine is used to treat motor symptoms of PD in the clinic but, unlike L-DOPA, does not induce LID (Pearce *et al*, 1998). It was therefore used as a dopaminergic control drug to

L-DOPA in the current chapter. Further description of bromocriptine's affinity for dopamine receptors is provided in section 6.4

All experiments used rats with MFB lesions as this is the lesion model in which previous studies have demonstrated disrupted synaptic plasticity following LID onset (Picconi *et al*, 2003). Furthermore, Lister Hooded rats were used over Sprague Dawley rats as the cohorts used in this chapter were also utilised in operant experiments where Lister Hooded rats were more appropriate.

6.2. Experiment 6 (part I): The effect of dopamine denervation and chronic L-DOPA treatment on maintenance of a previously acquired motor skill

6.2.1. Introduction

Whilst plasticity in the dopaminergic system has been suggested to play a role in motor skill acquisition (Luft *et al*, 2004; Yin *et al*, 2009; Wächter *et al*, 2010), synaptic reorganization does not appear to be crucial for the maintenance of motor skills (Wächter *et al*, 2010). Chronic L-DOPA, which induces LID in the majority of MFB lesion rats (Winkler *et al*, 2002), was therefore hypothesised to not affect rats' ability to maintain a previously learned motor skill. To test this hypothesis, rats that had been pre-trained on the staircase task and later given unilateral MFB lesions and chronic saline or L-DOPA treatment were compared in their performance on the staircase task. In line with previous publications (Wishaw *et al*, 1997) it was hypothesised that dopamine loss would impair performance on the staircase task when rats used their contralateral paw. However, chronic L-DOPA was not expected to further affect performance.

6.2.1.2. Aims

The aim of the experiment was to test whether dopamine denervation and chronic L-DOPA treatment affected pre-trained rats' ability to maintain their ability to retrieve sucrose pellets from staircase boxes

6.2.2. Methods

6.2.2.1. Experimental design

Female Lister hooded rats that were also used in the LCRT experiment described in section 7.3 were utilised in the experiment. All rats were pre-trained on the LCRT task and in

staircase boxes before a subgroup of the rats was given unilateral 6-OHDA lesions to the MFB. Rats were then chronically treated with saline or L-DOPA. Following one week food restriction, rats were tested in staircase boxes that were bilaterally baited with three sucrose pellets per step (Figure 6.1).

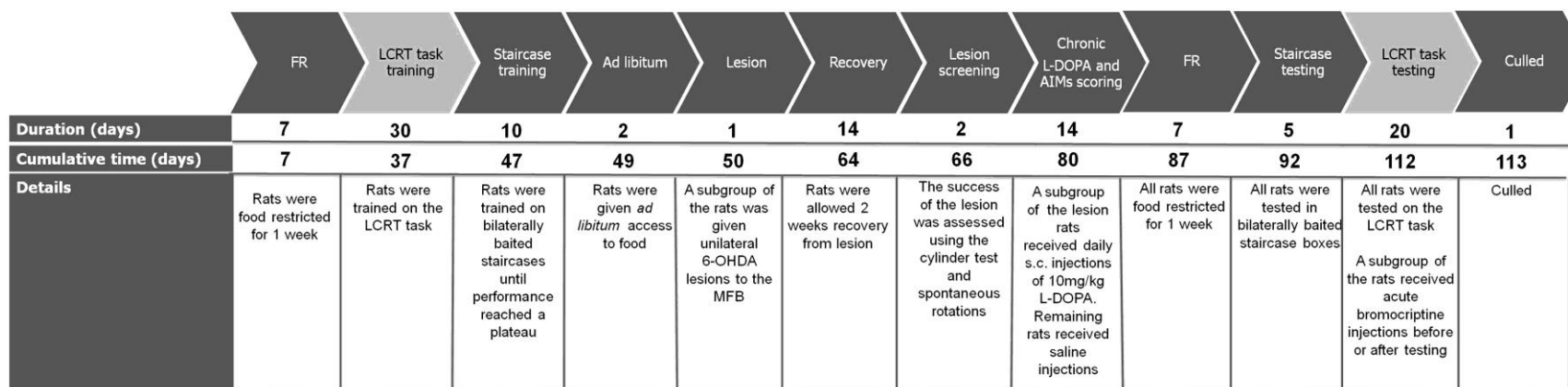


Figure 6.1. The experimental design used in Experiment 6. Grey boxes indicate testing reported further in section 7.3. The figure specifies both the duration of the individual testing phases, and the overall number of days in the experiment. AIMs=Abnormal involuntary movements, FR=Food restriction, LCRT=Lateralised choice reaction time task, MFB=Medial forebrain bundle.

6.2.2.2 Procedure

Forty-eight female Lister Hooded rats were pre-trained on the LCRT task for one month (section 2.7.2.2) and in the staircase boxes (section 2.4.7) for 10 days before 38 rats were given unilateral 6-OHDA lesions to the MFB (section 2.2.2). Following two weeks recovery, rats' lesion extent was assessed using the cylinder test and spontaneous rotations (sections 2.4.4 and 2.4.6.3). All intact and 26 of the lesion rats were then chronically treated with 1 ml/kg saline, and the remaining 12 lesion rats with 10 mg/kg L-DOPA for two weeks. AIMs were scored in

accordance with the previously described protocol (section 2.5). Only lesion rats treated with L-DOPA were scored for AIMs. This decision was based on the fact that LID is induced by chronic L-DOPA or D1 agonists, and is therefore not observed following only saline treatment. This was also confirmed in Experiment 10 where AIMs scoring of both saline and L-DOPA treated rats was conducted by a blinded scorer. Following induction of stable AIMs, rats were food restricted for before being tested in staircase boxes bilaterally baited with three pellets per step for five days.

During staircase testing rats were placed in the same staircase boxes they had been trained in and were allowed 15 min to reach for sucrose pellets, after which they were returned to their home cages. At the end of each session, the number of pellets remaining on each step was recorded to provide a measurement of the number of pellets the rats had successfully retrieved. In addition, the number of displaced pellets was counted as an indicator of attempted but failed retrievals, and the furthest away step the rats was been able to access was used as an indicator of reaching ability. A displaced pellet was defined as a pellet that was pushed from a higher step to a lower step. For example, if a step had been baited with three pellets, but five pellets were located on it at the end of staircase testing, two of these pellets must have been pushed there from an above step and were therefore defined as displaced.

At the end of testing, rats were used in an operant experiment (section 7.3) and thereafter perfused (section 2.8). Immunohistochemical staining was conducted as outlined in 2.10 and TH+ cell bodies were counted in the SNc and VTA (section 2.11) to confirm a lesion induced dopamine denervation.

6.2.2.3. Statistical analysis

The effect of the lesion on the number of TH+ cell bodies in the SNc and VTA was analysed using two separate types of analyses. First, the presence of a lesion in the SNc or VTA was confirmed by comparing the raw cell counts in the ipsilateral and contralateral hemisphere using a t-test. For this analysis, the SNc and VTA were analysed separately. Once the presence of a lesion had been confirmed, an ANOVA was used to test whether there was a difference in (i) the relative extent of the experimental groups' dopamine denervation, and (ii) the loss of dopaminergic cell bodies observed in the SNc versus the VTA. The ANOVA used the percentage loss of TH+ cell bodies observed in the lesion SNc and VTA as input data and included Region as a within-subject variable, and Group as a between-subject variable.

Baseline staircase data was analysed in a repeated measures ANOVA with Side as within-subject factor and Group as between-subject factor. Staircase data was analysed using a repeated measures ANOVA with Side and Day as within-subject factors and Group as between-subject factors. Significant between-factor differences were analysed using a Scheffe post-hoc test, whereas significant interactions were analysed using a test of simple effects.

6.2.3. Results

6.2.3.1. Lesion & Drug Effects

The success of the lesion was demonstrated by a significant reduction in the number of dopaminergic cell bodies observed in the ipsilateral compared to the contralateral SNc ($t_{37}=23.73$, $p<0.001$) and VTA ($t_{37}=6.99$, $p<0.001$). An analysis of the rats' percentage loss in their lesion relative to their intact hemisphere demonstrated that the lesion induced greater dopamine denervation in the SNc than in the VTA (Region: $F_{1,36}=37.33$, $p<0.001$), but that the two groups' did not differ in the extent of their dopamine loss (Group: $F_{1,36}=1.56$, n.s.; Group x Region: $F_{1,36}=0.59$, n.s.).

The unilateral dopamine loss was reflected in a pronounced ipsilateral bias in rats' spontaneous rotations (Figure 6.2C) and in their performance on the cylinder task (Figure 6.2D). There was no difference between the ipsilateral bias exhibited by the lesion groups on either of these motor tests (Spontaneous rotations: $F_{1,35}=0.13$, n.s.; Cylinder test: $U=131.5$, n.s.), suggesting that the lesion had a similar effect on motor function on all lesion groups.

Following chronic L-DOPA treatment, the lesion L-DOPA group exhibited high and stable AIMs scores in response to acute L-DOPA administration (Figure 6.2E).

6.2.3.2. Staircase data

Pre-lesion performance. To demonstrate that there was no difference in the groups' baseline performance three analyses were conducted; analysing the number of retrieved pellets, furthest step reached, and the number of displaced pellets respectively. There was no difference between the overall number of pellets the number of pellets the groups retrieved, the furthest step they could reach, or the overall number of pellets they displaced at baseline (Group: max. $F_{2,45}=1.17$, n.s.). Neither was there a difference in their ipsilateral bias on the above-mentioned measurements (Side: max. $F_{1,45}=0.70$, n.s.; Side x Group: max. $F_{2,45}=0.46$, n.s.).

Post-lesion performance: Pellets Retrieved. The two lesion groups retrieved significantly fewer pellets than intact controls when using their contralateral forelimb but

there was no effect of L-DOPA treatment on the lesion rats' performance (Figure 6.3A-B; Side: $F_{1,45}=57.64$, $p<0.001$; Side x Group: $F_{2,45}=21.56$, $p<0.001$; Simple Effect: $\min.F_{1,45}=64.53$, $p<0.001$). While most rats' performance was stable over the course of testing (Day: $F_{4,180}=1.13$, n.s.), the saline treated lesion group showed a small but significant increase in the number of pellets they could retrieve using their ipsilateral paw (Side x Day x Group: $F_{8,180}=2.37$, $p<0.05$; Simple Effect: $F_{4,42}=4.16$, $p<0.05$), as well as a small but significant

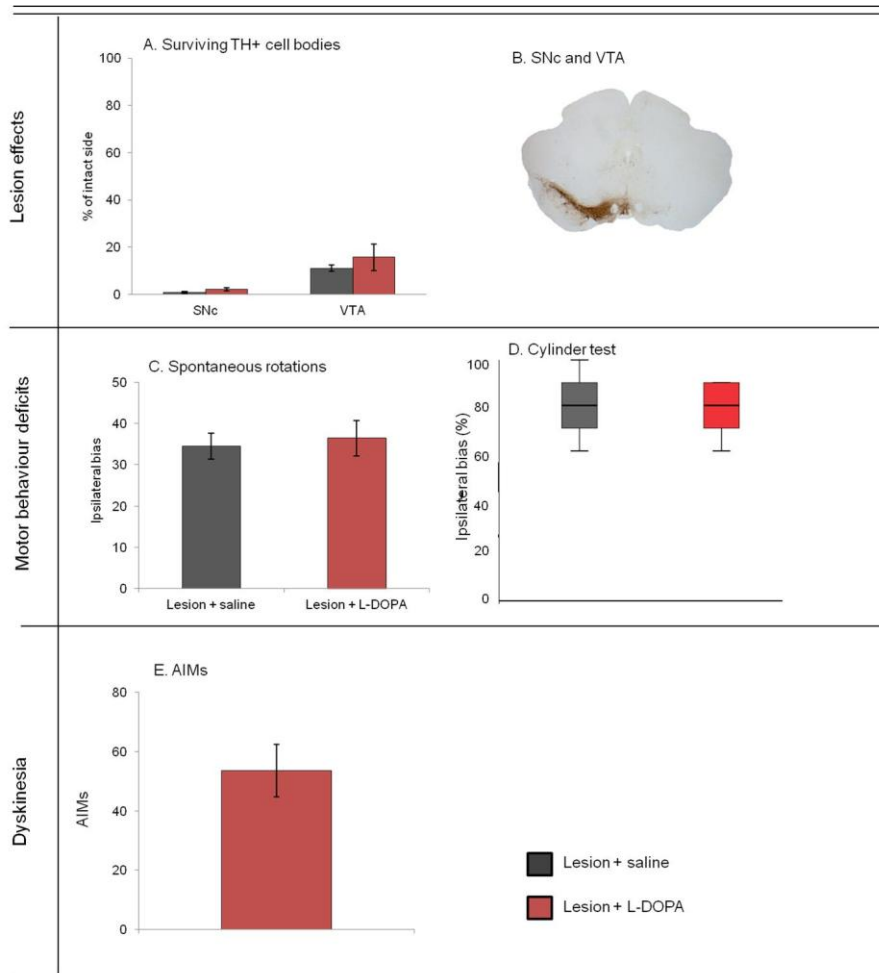


Figure 6.2. Surviving dopaminergic cell bodies in the SNc and VTA of rats that were L-DOPA naive or L-DOPA treated, expressed as a percentage of the intact side (A), together with the a photo showing the loss of TH+ cell bodies in the SNc and VTA in one lesion rat included in the experiment (B), the ipsilateral bias exhibited by lesion rats that were later L-DOPA naive or L-DOPA treated in the spontaneous rotations (C) an cylinder (D) tests, and the cumulative AIMs s scores for the lesion L-DOPA treated rats following chronic L-DOPA treatment (E). It is noted that all rats were L-DOPA naive at the time of lesion and the motor behavioural tests The bars charts (A, C) show the mean and standard error of the mean. The box plot (D) shows the median and the interquartile ranges. SNc=Substantia nigra pars compacta; TH=Tyrosine hydroxylase; VTA=Ventral tegmental area. * $p<0.001$**

decrease in the number of pellets they could retrieve using their contralateral paw (Simple Effect: $F_{4,42}=4.75$, $p<0.01$).

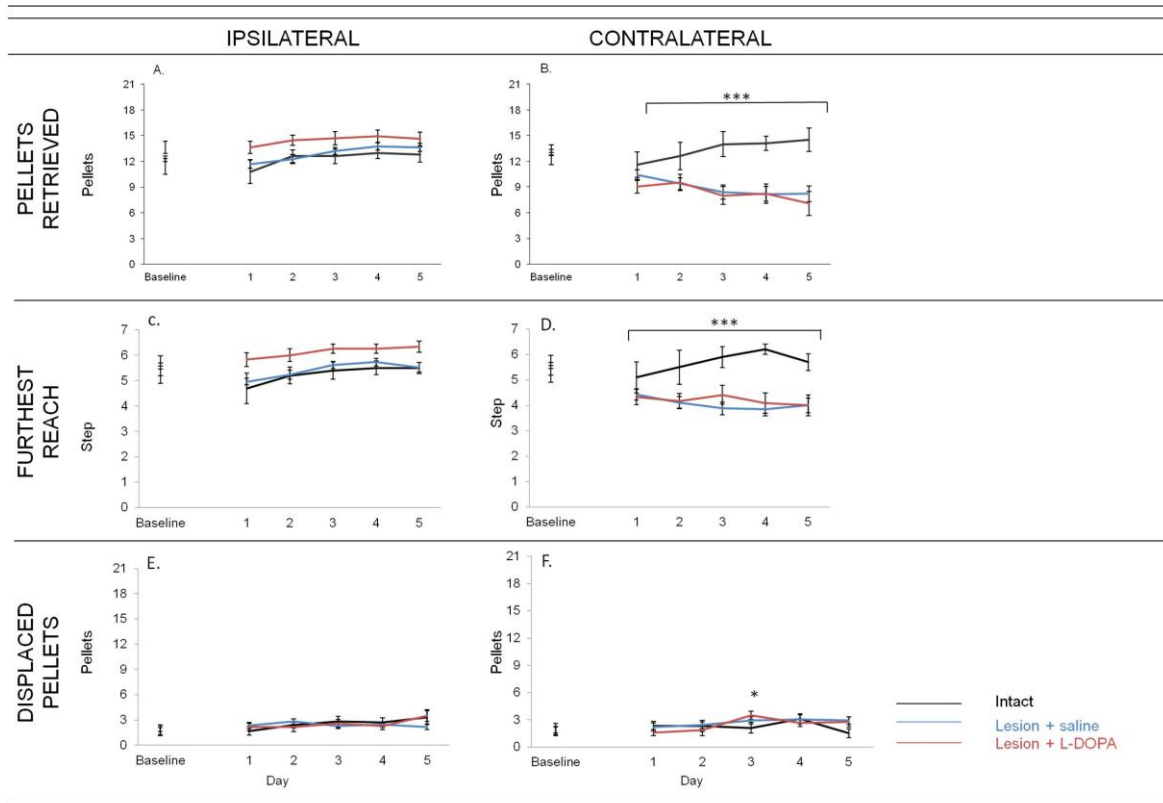


Figure 6.3. The number of pellets retrieved from staircase boxes (A-B), the furthest step pellets were retrieved from (C-D), and the number of pellets displaced (E-F) by drug naïve intact ($n=10$), lesion drug naïve ($n=26$), and lesion L-DOPA treated rats ($n=12$) rats. * $p<0.05$, *** $p<0.001$

Post-lesion performance: Furthest Step Reached. Both lesion groups could reach further when using their ipsilateral than when using their contralateral forelimb (Figure 6.3C-D; Side x Group: $F_{2,45}=23.36$, $p<0.001$; Simple Effect: min. $F_{1,45}=62.32$, $p<0.001$). There was an overall change in ipsilateral reaching ability over the course of testing which was driven by a slight improvement in the performance of the intact, but not lesion, rats (Side x Day: $F_{4,180}=3.04$, $p<0.05$; Simple Effect: $F_{4,42}=4.20$, $p<0.01$). Overall, the intact and the lesion L-DOPA treated groups could both reach steps located further away than the lesion saline treated group could (Group: $F_{2,45}=4.54$, $p<0.05$).

Post-lesion performance: Displaced Pellets. There was no difference between the number of pellets that were displaced by the three groups, regardless of whether the ipsilateral or contralateral forelimb was used (Figure 6.3E-F; Side: $F_{1,45}=0.07$, n.s.; Side x Group: $F_{2,45}=0.71$, n.s.). While the overall number of displaced pellets fluctuated over the

course of testing, visual inspection of the graphs suggested this was merely due to noise in the data (Day: $F_{4,180}=2.66$, $p<0.05$; Side x Day: $F_{4,180}=1.26$, n.s.; Day x Group: $F_{8,180}=0.81$, n.s.; Side x Group x Day: $F_{8,180}=1.29$, n.s.; Group: $F_{2,45}=0.12$, n.s.).

6.3. Experiment 7: The effect of dopamine denervation and chronic L-DOPA treatment on the ability for *de novo* motor learning

6.3.1. Introduction

Based on data demonstrating synaptic reorganization in the motor cortex and dorsal striatum following learning of a novel motor skill (Luft *et al*, 2004; Yin *et al*, 2009; Wächter *et al*, 2010), it was hypothesized that the aberrant plasticity occurring in the dopaminergic system following LID onset (Picconi *et al*, 2003) would impair *acquisition* of a novel motor skill. To test this hypothesis, the rate with which MFB lesion chronically treated with saline or L-DOPA learned to retrieve sucrose pellets from staircase boxes when no pre-training had been given was compared. In line with previous publications (Whishaw *et al*, 2007) it was hypothesised that unilateral MFB lesion cause lesion rats to retrieve fewer pellets than intact controls when using their contralateral paw. Furthermore, it was hypothesised that lesion rats treated with L-DOPA, which causes LID onset in the vast majority of MFB lesion rats (Winkler *et al*, 2002) would show a slower rate of learning on the staircase task than lesion rats treated with saline.

6.3.2. Aims

The aim of the experiment was to study the effect of chronic L-DOPA treatment and LID onset on MFB lesion rats' ability to learn to retrieve sucrose pellets in staircase boxes.

6.3.2. Methods

6.3.2.1. Experimental design

While written up as the second staircase experiment, the current section describes the first staircase experiment conducted in this thesis project. The rat cohort described was used both in the staircase testing described in this section, and in an operant pilot study (not reported). As part of the operant pilot study, all rats were given 3-4 days of magazine and lever press training before a subgroup received unilateral MFB lesions. After recovery, the lesion group was chronically treated with saline or L-DOPA and thereafter tested on an operant task. Following operant testing, a second AIMs scoring session took place to ensure the L-DOPA treated rats still exhibited AIMs. All rats were thereafter tested for 20 days in unilaterally

baited staircase boxes (Figure 6.4). Thus, it is noted that the current experiment and Experiment 6 did not only differ in terms of whether or not rats were pre-trained on the staircase task but also in the type and length of operant training which they underwent.

6.3.2.2. Procedure

Thirty-five female Lister hooded rats were given 3-4 days magazine training in two-lever operant boxes before a subgroup (n=20) was given unilateral lesion lesions of the MFB (section 2.2.2). Following two weeks recovery, lesion extent was assessed using the cylinder test and spontaneous rotations (sections 2.4.4 and 2.4.6.3). A subgroup of the lesion rats (n=9) was chronically treated with 10 mg/kg L-DOPA, daily, for two weeks whilst the remaining rats received saline injections. AIMS were scored in accordance with the previously described protocol (section 2.5). Only lesion rats treated with L-DOPA were scored for AIMS. This decision was based on the fact that LID is by definition not induced by saline, something which was also confirmed in Experiment 10 where AIMS scoring of both saline and L-DOPA treated rats was conducted.

Following induction of stable AIMS, rats were tested on a simple discrimination task (section 2.7.1.2) before being tested in staircase boxes. The L-DOPA group was given a further injection/AIMS scoring session between operant and staircase testing to ensure they still exhibited a dyskinetic response to 10 mg/kg L-DOPA, which all rats did.

Staircase testing was conducted using the same protocol as described in section 6.2 but using unilaterally baited rather than bilaterally baited staircase boxes. The side of baiting alternated each day. Unilateral rather than bilateral baiting was used to force lesion rats to use their contralateral paw, thereby ensuring that the data reflected *de novo* learning rather than a decrease in the willingness to exert effort for a reward following 6-OHDA lesions as has previously been observed (Cousins & Salamone, 1994). The side which rats first began testing on (i.e. ipsilateral or contralateral to the lesion) was counterbalanced.

Following staircase testing, rats were tested on the 100 pellets test. Described in further detail in section 2.4.1, the test assessed the amount of time taken for rats to eat 100 freely available sucrose pellets and was used as an indicator of the rats' motivation to consume the sucrose pellets.

At the end of testing, all rats were culled (2.8). The tissue was used for immunohistochemical staining as outlined in section 2.10 and TH+ cell bodies were counted in the SNc and VTA (2.11) to confirm the success of the lesion. Striatal tissue was intended to be used for

molecular studies but as it was not within the timeframe of the thesis project to optimise the necessary protocols such data are not reported.

6.3.2.3. Statistical analysis

The effect of the lesion on the number of TH+ cell bodies in the SNc and VTA was analysed using two separate types of analyses. First, the presence of a lesion in the SNc or VTA was confirmed by comparing the raw cell counts in the ipsilateral and contralateral hemisphere using a t-test. For this analysis, the SNc and VTA were analysed separately. Once the presence of a lesion had been confirmed, an ANOVA was used to test whether there was a difference in (i) the relative extent of the experimental groups' dopamine denervation, and (ii) the loss of dopaminergic cell bodies observed in the SNc versus the VTA. The ANOVA used the percentage loss of TH+ cell bodies observed in the lesion SNc and VTA as input data and included Region as a within-subject variable, and Group as a between-subject variable.

Baseline staircase performance was measured by analysing performance on the final testing day with Side as a within-subject factor and Group as a between-subject factor. Post-lesion staircase performance was analysed in a repeated measures ANOVA with Side and Day as within-subject factors and Group as between-subject factors. Data from the 100 Pellets Test were analysed using a one-way ANOVA with Time as a within-subject factor and Group as a between-subject factor. Significant between-subject differences were analysed using a Scheffe post-hoc test whereas significant interactions were analysed using a test of simple effects.

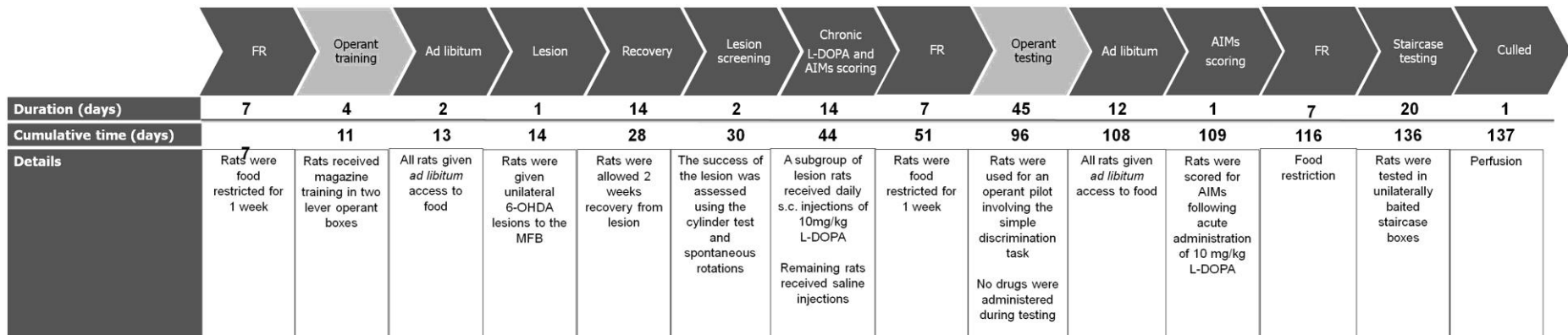


Figure 6.4. A graphical representation of the experimental design of Experiment 7. The figure specifies both the duration of the individual testing phases, and the overall number of days in the experiment. Grey boxes indicate testing undertaken for a pilot experiment not reported in the thesis. AIMs=Abnormal involuntary movements, FR=Food restricted, MFB=Medial forebrain bundle, s.c.=subcutaneous

6.3.3. Results

6.3.3.1. Lesion & Drug Effects

The lesion induced a profound loss of dopaminergic cell bodies in the ipsilateral SNc (Figure 6.5A; $t_{24}=14.93$, $p<0.001$) and the VTA ($t_{24}=12.35$, $p<0.001$). A further analysis demonstrated that the percentage TH loss was greater in the SNc than in the VTA (Region: $F_{1,23}=54.71$, $p<0.001$) and that the denervation was similar between the groups (Group: $F_{1,23}=1.18$, n.s.; Region x Group: $F_{1,23}=0.25$, n.s.).

The success of the lesion was also reflected in an ipsilateral bias in the rats' spontaneous rotations (Figure 6.5C) and in the cylinder test (Figure 6.5D). There was no difference between the groups' ipsilateral bias on either of these motor tests (Spontaneous rotations: $F_{1,24}=0.03$, n.s.; Cylinder test: $U=72.00$, n.s.). Chronic L-DOPA treatment induced LID, characterised by stable AIMs in all L-DOPA treated rats (Figure 6.5E).

6.3.3.2. Staircase data

Pellets retrieved. Lesion rats retrieved significantly fewer pellets than intact controls but there was no difference between the overall number of pellets retrieved by lesion rats treated with saline and L-DOPA (Figure 6.6A-B; Group: $F_{2,32}=8.84$, $p<0.01$). While lesion rats retrieved fewer pellets than intact controls using their ipsilateral paw on days 5, 6, and 9 of testing, the lesion induced deficit was most pronounced when rats had to use their contralateral forelimb to retrieve pellets. When using their contralateral forelimb, lesion rats were impaired from the second until the last day of testing (Side x Day x Group: $F_{18,288}=2.11$, $p<0.05$; Simple Effect: min. $F_{2,32}=3.48$, $p<0.05$).

Furthest Step Reached. Overall, intact rats could reach steps located further away than lesion rats treated with L-DOPA while there was no overall difference between intact rats and lesion rats treated with saline (Figure 6.6C-D; Group: $F_{2,32}=7.31$, $p<0.01$). However, a general lesion deficit did present itself when rats used their contralateral forelimb, with both lesion groups but not the intact rats exhibiting a significant ipsilateral bias throughout the course of testing (Side x Day x Group: $F_{18,288}=2.01$; Simple Effect of Side: min. $F_{1,32}=2.63$, $p<0.05$). There was also a difference in the degree of their improvement over the course of testing. When using the ipsilateral forelimb, lesion L-DOPA treated rats could initially not reach as far as the other two groups but due to a rapid learning curve their ipsilateral performance aligned with that of the intact and saline treated lesion rats at the end of testing. When using their contralateral forelimb, both intact and saline treated lesion rats showed a significant improvement over the course of testing (Simple Effect: min $F_{9,24}=2.63$, $p<0.05$).

While there was a trend towards a similar improvement in the lesion L-DOPA treated group this did not reach statistical significance ($p=0.057$).

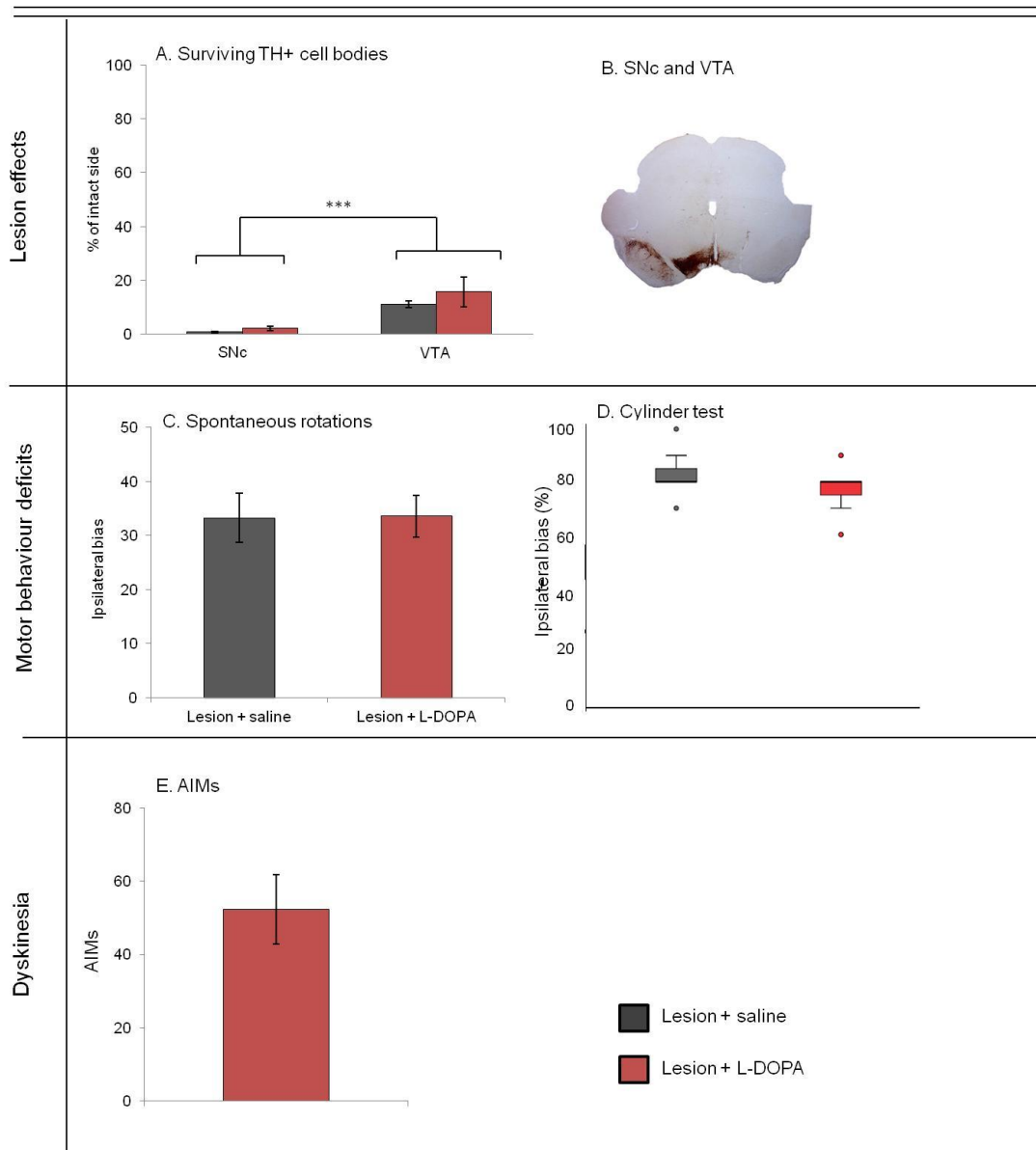


Figure 6.5. Surviving dopaminergic cell bodies in the SNc and VTA (A), expressed as a percentage of the intact side, together with the ipsilateral bias on the spontaneous rotations (C) and cylinder (D) tests of lesion rats that were later chronically treated with saline (n=11) or L-DOPA (n=9). The graph also shows the average cumulative AIM scores from the lesion L-DOPA groups' last two dyskinesia scoring sessions (E). It is noted that all rats were L-DOPA naive at the time of lesion and the motor behavioural tests. AIMs=Abnormal involuntary movements, SNc=Substantia nigra pars compacta, TH=Tyrosine hydroxylase, VTA=Ventral tegmental area. * $p<0.001$**

Displaced Pellets. The number of displaced pellets was similar for all three groups, when measuring ipsilateral as well as contralateral performance (Figure 6.6E-F; Group: $F_{2,32}=1.51$, n.s.; Side: $F_{1,32}=0.06$, n.s.; Side x Group: $F_{2,32}=1.00$, n.s.; Side x Day x Group: $F_{18,288}=1.57$, n.s.). While there were some fluctuations in the number of pellets that were displaced when rats used their contralateral paws, this was not specific to any experimental group and visual inspection of the data suggested that it was due to noise in the data (Side x Day: $F_{18,288}=3.13$, $p<0.01$; Simple Effect: $F_{9,24}=2.62$, $p<0.05$; Day: $F_{9,288}=1.80$, n.s.; Day x Group: $F_{18,288}=0.59$, n.s).

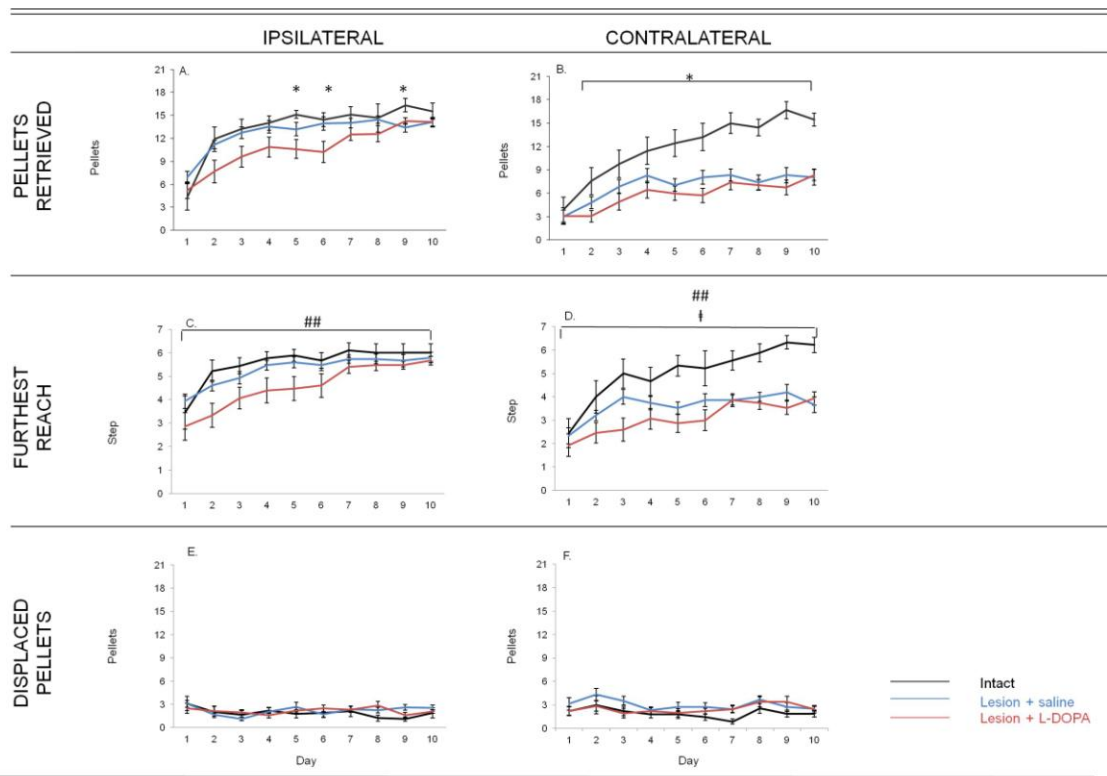


Figure 6.6. The number of pellets obtained from staircase boxes (A-B), the furthest step pellets were retrieved from (C-D), and the number of pellets displaced (E-F) by intact (n=15), lesion drug naïve (n=11), and lesion rats chronically treated with L-DOPA (n=9) rats. *Significant difference between intact rats and lesion rats treated with saline and L-DOPA. #Significant difference between intact and lesion rats treated with L-DOPA, ‡Significant difference between lesion saline treated and lesion L-DOPA treated rats' contralateral and ipsilateral performance. * $p<0.05$, ## $p<0.01$, ‡ $p<0.01$

6.3.3.3. 100 Pellets Test

When given the opportunity to consume 100 freely available sucrose pellets, the two lesion groups took significantly longer time than the intact controls to consume all pellets (Figure

6.7). There was no further effect of L-DOPA treatment on lesion rats' performance (Group: $F_{2,33}=5.28$, $p<0.05$).

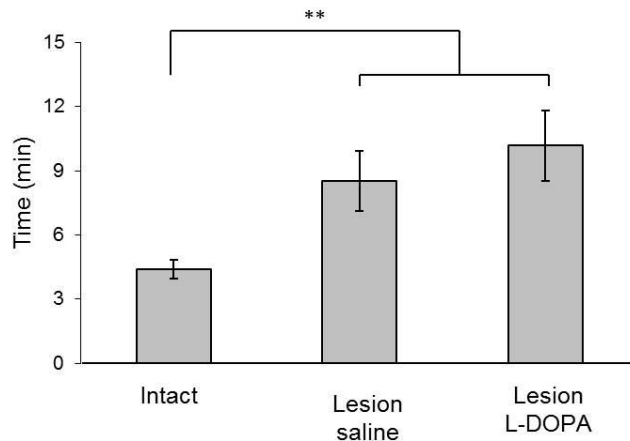


Figure 6.7. The time (min) taken for intact rats ($n=15$), and lesion rats chronically treated with saline ($n=11$) or L-DOPA ($n=9$) to consume 100 freely available sucrose pellets. Lesion rats took significantly longer time than intact controls to consume the available pellets, but there was no effect of L-DOPA treatment. **** $p<0.01$**

6.4. Experiment 8 (part I): The effect of dopamine denervation and chronic L-DOPA or bromocriptine treatment on the ability for *de novo* motor learning

6.4.1. Introduction

Data from Experiment 7 suggested impaired acquisition of a novel motor skill following chronic L-DOPA treatment to lesion rats. More precisely, chronic L-DOPA treatment was associated with impaired ability to retrieve sucrose pellets from steps located further away, i.e. pellets located on steps that were more difficult for rats to reach. While significant, the impairment was subtle. In addition to attempting to replicate the phenomenon, the current experiment therefore also increased the number of sucrose pellets located on each staircase step from three to four pellets in an attempt to make the test more sensitive and thereby increase the likelihood of observing a difference between lesion rats treated with saline or L-DOPA.

Chronic L-DOPA induces LID in the majority of MFB lesion rats (Winkler *et al*, 2002), a condition that has been associated with disrupted synaptic plasticity in the cortico-striatal pathway (Picconi *et al*, 2003). Given the relationship between striatal synaptic plasticity and learning new motor skills (e.g. Luft *et al*, 2004) it was hypothesized that the impaired ability to reach sucrose pellets on steps located further away following L-DOPA treatment in Experiment 7 had been associated with LID onset. Ideally, this hypothesis would be tested by comparing the performance of lesion rats that did or did not developed LID following chronic L-DOPA treatment. However, previous publications have demonstrated that only a small proportion of MFB lesion rats do not develop LID in response to chronic L-DOPA (Winkler *et al*, 2002) and none of the lesion L-DOPA treated rats in Experiment 7 remained non-dyskinetic following L-DOPA treatment. Conversely, LID is not induced chronic treatment with the dopamine agonist bromocriptine (Pearce *et al*, 1998; Lieberman & Goldstein, 1985; Pearce *et al*, 1998; Kvernmo *et al* 2006; Stern, 2008) which primarily binds to D2 receptors (Table 6.1; Kvernmo *et al*, 2006). To further test the hypothesis that the previously observed difference between lesion rats treated with saline or L-DOPA was due to LID onset rather than mere exposure to dopaminergic treatment, bromocriptine was used as a control drug in the current experiment.

Table 6.1. Bromocriptine’s binding affinities (K_i in nmol/L) for dopaminergic and serotonergic receptors. Adapted from Kvernmo *et al* (2006).

| D1type | D2type | D ₂ | D ₃ | D ₄ | D ₅ | 5-HT _{1B} | 5-HT _{1B} | 5-HT _{1D} | 5-HT _{2A} | 5-HT _{2B} | 5-HT _{2C} |
|--------|--------|----------------|----------------|----------------|----------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| 1659 | 12.2 | 2.5 | 12.2 | 59.7 | 1691 | 12.9 | 354.8 | 10.7 | 107.2 | 56.2 | 741.3 |

In addition to comparing the performance of lesion rats treated with L-DOPA and bromocriptine, the experiment also expanded on the previous findings by testing whether chronic L-DOPA also affected intact rats’ performance. As L-DOPA does not induce LID in intact rats, it was hypothesized that any effect of L-DOPA would be restricted to the lesion group. Similarly, because bromocriptine does not induce LID when administered to lesion rats as a monotherapy (Pearce *et al*, 1998) it was further hypothesised that no effect would be observed by bromocriptine treatment.

6.4.2. Aims

The aim of the experiment was to (i) replicate the findings in Experiment 7, which suggested that chronic L-DOPA impaired lesion rats’ learning on the staircase task, and (ii) test whether chronic bromocriptine also affected staircase task learning in lesion rats.

6.4.2. Methods

6.4.2.1. Experimental design

The cohort reported here was also used in an operant experiment described in section 7.4. All rats underwent LCRT task training before a subgroup was given unilateral 6-OHDA lesions to the MFB. Intact and lesion rats were then chronically treated with saline, bromocriptine, or L-DOPA. Following one week of food restriction, rats were tested in unilaterally baited staircase boxes for 20 days (Figure 6.8). Further details on the operant testing the rats underwent are provided in section 7.4. It is caveated that because rats were used in more than one experiment, in order to reduce the overall number of licensed procedures that were conducted and the number of rats that were sacrificed as part of the thesis project, there are some differences in the operant testing that the rats in this and Experiment 7 underwent.

6.4.2.2. Procedure

Eighty-five female Lister hooded rats were pre-trained on the LCRT task for 1 month (section 2.7.2.2) before a subset of rats (n=55) was given unilateral lesion lesions to the MFB (section 2.2.2). Following two weeks recovery, the lesion extent was assessed using the cylinder test and spontaneous rotations (sections 2.4.4 and 2.4.6.3) and rats were then chronically treated with their respective drug: 1 mg/kg saline (intact, n=19; lesion, n=15), 2.5 mg/kg bromocriptine (intact, n=10; lesion, n=15) or 10 mg/kg L-DOPA (intact, n=10; lesion, n=25) once daily for 3 weeks. AIMs were scored in accordance with the previously described protocol (section 2.5). Only lesion rats treated with L-DOPA or bromocriptine were scored for AIMs. This decision was based on the fact that LID by definition does not occur in lesion saline treated rats. This was also demonstrated in Experiment 10 where AIMs scoring of lesion rats treated with saline or L-DOPA was conducted by a scorer blinded to the drug treatments the rats received. In addition to AIMs scoring, the effect of chronic bromocriptine treatment was assessed every third day of treatment by measuring the rats' net rotational bias in automated rotometers (section 2.4.6.1) for 90 min post-injection. The effect of L-DOPA was not assessed using rotometers as previous authors have demonstrated that L-DOPA induced rotations are not a good indicator for LID (Lundblad *et al*, 2002) and because of limited availability of rotometers.

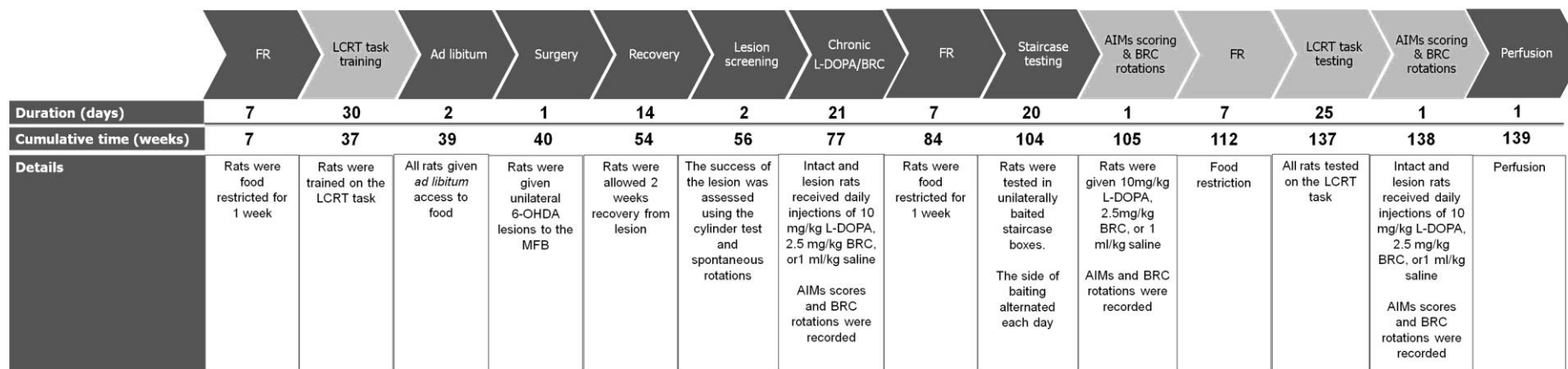


Figure 6.8. A graphical representation of the experimental design of Experiment 8. Grey boxes represent testing reported in section 7.4. The figure specifies both the duration of the individual testing phases, and the overall number of days in the experiment. AIMS=Abnormal involuntary movements, BRC=Bromocriptine, FR=Food restriction, LCRT task=Lateralised choice reaction time task, MFB=Medial forebrain bundle.

It is brought to the reader's attention that, unlike Experiment 7, a subset (n=4) of lesion rats in the current experiment remained non-dyskinetic following L-DOPA treatment. In this thesis, a rat was defined as dyskinetic if she exhibited severity AIMS >1 on the orolingual, axial, or forelimb AIMS subtypes during any of the 1 min AIMS scoring time points. This classification is in line with criteria used in previous rat dyskinesia studies (Lindgren *et al*, 2010) and has been shown to distinguish between rats with high and low striatal levels of dyskinesia markers such as phosphorylated ERK1/2 (Westin *et al*, 2007), FosB, and PDyn mRNA (Andersson *et al*, 1999). In addition, following acute L-DOPA administration, rats classified as non-dyskinetic using this cut-off have been shown to have lower levels of extracellular DOPA in the SNc and striatum than dyskinetic controls (Lindgren *et al*, 2010), and, unlike dyskinetic rats, do not show an angiogenic response to L-DOPA treatment (Westin *et al*, 2006)

At the end of drug treatments, rats were food restricted for one week. They were thereafter tested in unilaterally baited staircase boxes for 20 days in the same manner as described in 7.2 but with four, instead of three, sucrose pellets per step. Because the difference observed between lesion drug naïve and lesion L-DOPA rats in Experiment 7 had been subtle, the number of pellets per step was increased to increase the sensitivity of the test and therefore also the likelihood of observing a significant difference between the groups. As in section Experiment 7, the side of baiting alternated daily and the side rat began testing on (i.e. ipsilateral or contralateral to the lesion) was counterbalanced.

At the end of testing, the rats were culled using an overdose of Euthatal and decapitated. Rats' striata were dissected out and their hindbrains postfixed overnight in 4% PFA before being transferred to a 25% sucrose solution (2.9). Immunohistochemical staining was conducted as outlined in section 2.10 and nigral TH+ cell bodies counted in each hemisphere (section 2.11) to confirm a lesion induced dopamine denervation. While the striatal tissue was originally intended for Western blotting it was not possible to refine the appropriate Western blotting protocols and conduct molecular work within the time frame of the thesis project.

6.4.2.3. Statistical analysis

The effect of the lesion on the number of TH+ cell bodies in the SNc and VTA was analysed using two separate types of analyses. First, the presence of a lesion in the SNc or VTA was confirmed by comparing the raw cell counts in the ipsilateral and contralateral hemisphere using a t-test. For this analysis, the SNc and VTA were analysed separately. Once the presence of a lesion had been confirmed, an ANOVA was used to test whether there was a difference in (i) the relative extent of the experimental groups' dopamine denervation, and (ii) the loss of dopaminergic cell bodies observed in the SNc versus the VTA. The ANOVA used the percentage loss of TH+ cell bodies observed in the lesion SNc and VTA as input data and included Region as a within-subject variable, and Group as a between-subject variable.

When analysing staircase data, Side and Day were used as within-subject factors, and Group as a between-subject factor. The change in bromocriptine induced rotations following chronic treatment was analysed using a one-way ANOVA. Rats that had begun testing on the ipsilateral and contralateral side respectively were pooled according to lesion and drug treatment and the following groups were generated: intact + saline (n=10), intact + L-DOPA (n=10), intact + bromocriptine (n=10), lesion + saline (n=15), lesion + L-DOPA (n=25),

lesion + bromocriptine (n=15). Four of the lesion L-DOPA treated rats did not develop LID following L-DOPA treatment. Data from Experiment 7 had not provided insight into whether the observed L-DOPA effect had been due to chronic L-DOPA or LID onset. Therefore, two analyses were conducted in the current section; one grouping all lesion L-DOPA treated rats together during statistical analysis and a second separating this group into a dyskinetic and non-dyskinetic group.

6.4.3. Results

6.4.3.1. Lesion & Drug effects

The lesion induced a significant loss of dopaminergic cell bodies in the ipsilateral SN (Figure 6.9A; $t_{54}=30.98$, $p<0.001$) and VTA ($t_{54}=31.02$, $p<0.001$). A further analysis demonstrated that there was a greater percentage loss of dopaminergic cell bodies in the ipsilateral SNc than in the ipsilateral VTA (Region: $F_{1,51}=26.43$, $p<0.001$) and that the dopamine denervation was similar between the groups (Group: $F_{3,51}=0.66$, n.s.; Region x Group: $F_{3,51}=0.39$, n.s.).

The lesion induced an ipsilateral bias on the spontaneous rotations and cylinder tests (Figure 6.9C-D) that did not differ between the three groups (Spontaneous rotations: $F_{2,52}=1.55$, n.s.; Cylinder test: $H(2)=0.74$, n.s.). The lesion group treated with L-DOPA exhibited high AIMs scores following chronic treatment (Figure 6.9E), something which was not observed in the lesion group treated with bromocriptine (Group: $F_{1,39}=16.97$, $p<0.001$). Chronic bromocriptine did, however, produce an increase in the net bias of rats' drug-induced rotations (Figure 7.16F; $F_{1,15}=12.39$, $p<0.01$).

Using a previously published cut-off for AIMs (Lindgren *et al*, 2010) all but four of the lesion L-DOPA rats were classified as dyskinetic. Conversely, AIMs induced by chronic bromocriptine treatment fell below the previously published cut-off line for dyskinetic rats and these rats were therefore considered non-dyskinetic. Bromocriptine treatment did, however, increase the net bias of bromocriptine-induced rotations as shown when comparing the first and last rotometer session (Figure 6.9C; $t_{15}=-3.9$, $p<0.01$).

6.4.3.2. Staircase data: L-DOPA treated rats treated as one group

While lesion rats were impaired relative to intact controls in the number of pellets they could retrieve using their contralateral paw (Group x Side: $F_{5,79}=13.92$, $p<0.001$; Simple Effect of Side: $\min.F_{1,79}=40.64$, $p<0.001$) and the furthest step they could reach on their contralateral side (Group x Side: $F_{5,79}=10.09$, $p<0.001$; Simple Effect of Side $\min.F_{1,79}=34.29$, $p<0.001$) there was no difference between the three intact groups on these measures. Similarly, while lesion rats treated with bromocriptine displaced overall more pellets than the remaining

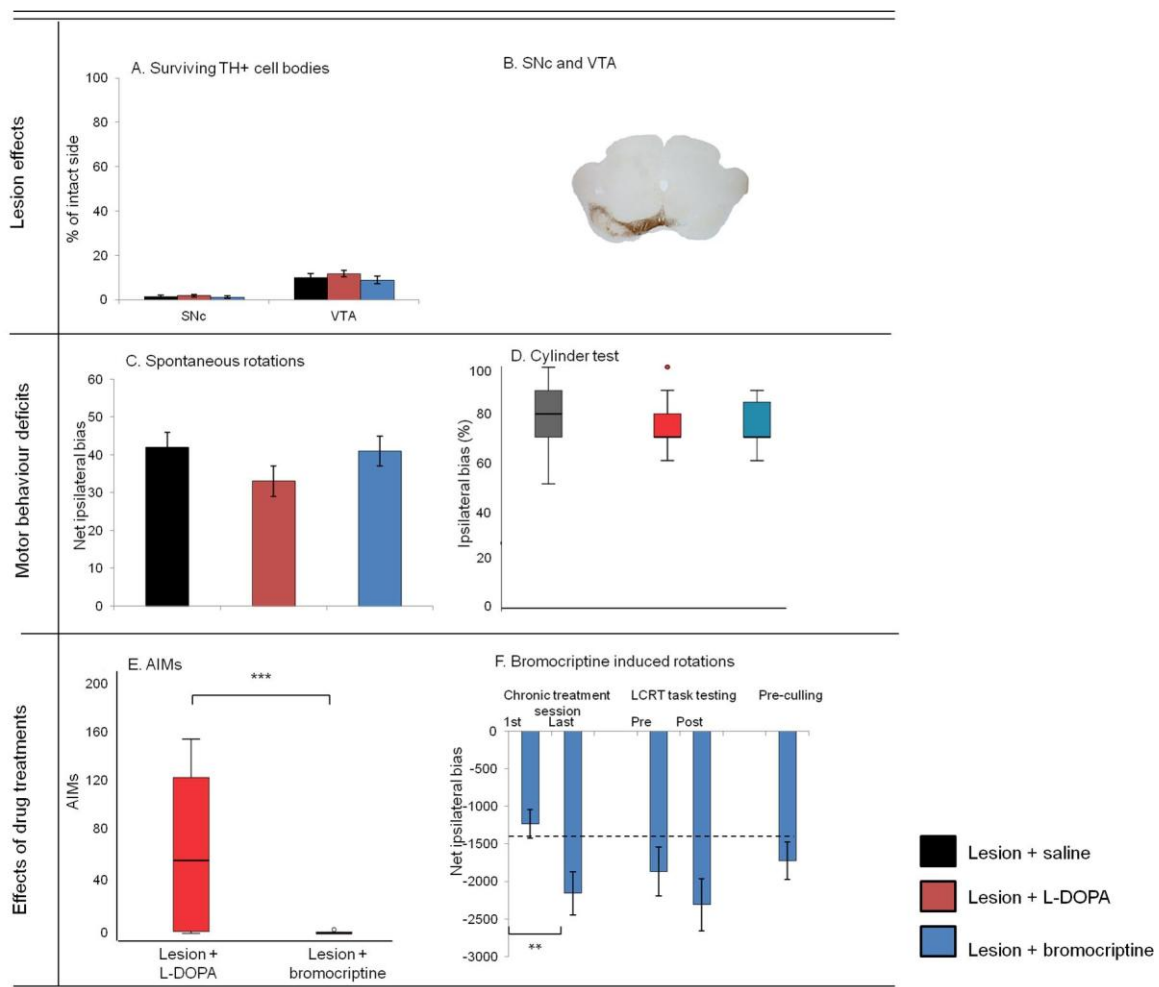


Figure 6.9. The figure shows the surviving dopaminergic cell bodies in the SNc and VTA, expressed as a percentage of the intact side (A), a photo showing the SNc and VTA denervation of a lesion rat included in the experiment (B), the ipsilateral bias exhibited on the spontaneous rotations (C) and cylinder tests (D), the AIMs scores (E) of lesion rats chronically treated with L-DOPA (n=25), and bromocriptine (n= 15), as well as net rotational bias of rotations following acute bromocriptine administration (C). AIMs=Abnormal involuntary movements, SNc=Substantia nigra pars compacta, TH=Tyrosine hydroxylase, VTA=Ventral tegmental area. **p<0.01, ***p<0.001

groups (Group x Side: $F_{5,79}=0.58$, n.s.; Group: $F_{5,79}=2.89$, $p<0.05$) there was no difference between the number of pellets displaced by the intact groups. Therefore, the data from the intact groups were pooled in the analyses presented below.

Pellets retrieved. The number of successfully retrieved pellets increased in all groups over the course of testing (Figure 6.10A-B; Day: $F_{9,729}=54.63$, $p<0.001$; Day x Group: $F_{27,729}=1.03$, n.s.). However, a significant contralateral deficit was observed in all lesion

groups. Relative to their ipsilateral performance, lesion rats treated with saline or L-DOPA were impaired throughout testing when using their contralateral forelimb side. A similar impairment was observed from the second until the last day testing in the lesion group that had been treated with bromocriptine (Side x Day x Group: $F_{27,729}=2.07$, $p<0.01$; Simple Effect: $\min.F_{1,81}=5.65$, $p<0.05$).

Furthest Step Reached. The lesion groups could not reach as far as the intact controls when using their contralateral paw. This contralateral deficit was statistically significant from the second until the last day of testing in all lesion groups (Figure 6.10C-D; Side x Day x Group: $F_{27,729}=1.76$, $p<0.05$; Effect of Side: $\min.F_{1,81}=8.99$, $p<0.01$).

Displaced Pellets. Overall, intact controls displaced fewer pellets than the lesion rats (Figure 6.10E-F; Group: $F_{3,81}=4.86$, $p<0.05$). While the number of pellets displaced by the rats fluctuated over the ten testing days (Day: $F_{9,729}=22.05$, $p<0.001$), visual inspection of the data suggested this was due to noise in the data rather than reflective of an improvement or worsening of performance.

6.4.3.2. Staircase data: lesion L-DOPA treated group divided into dyskinetic and non-dyskinetic rats

A second analysis was conducted in which the L-DOPA treated group was split into two cohorts depending on whether or not they were classified as non-dyskinetic using a previously published cut-off (Lindgren *et al*, 2007).

Pellets retrieved. Intact rats retrieved more pellets than lesion rats, but there was no overall difference between the four lesion groups (Figure 6.11A-B; Group: $F_{4,80}=8.90$, $p<0.001$). Lesion rats were impaired when using their contralateral paw (Side x Day x Group: $F_{36,729}=19.40$, $p<0.05$; Simple Effect of Side: $\min.F_{1,80}=4.38$, $p<0.05$). Intact rats showed day-to-day improvement in their ipsilateral as well as contralateral performance. Conversely, lesion rats treated with saline, bromocriptine, or rats that developed LID following L-DOPA treatment only showed a significant day-to-day improvement in their ipsilateral performance. Lesion rats that remained non-dyskinetic following L-DOPA treatment showed significant fluctuations in their contralateral performance. However, visual inspection of the data suggested this change in performance was due to random fluctuations and not a day-to-day improvement in their performance (Simple Effect of Day: $\min.F_{9,72}=2.30$, $p<0.05$).

Furthest step reached. Rats performance improved over the course of testing, as evidenced by a greater ability to reach steps located far away at the end versus the beginning of the testing period (Figure 6.11C-D; Day: $F_{9,720}=50.84$, $p<0.001$). While intact rats'

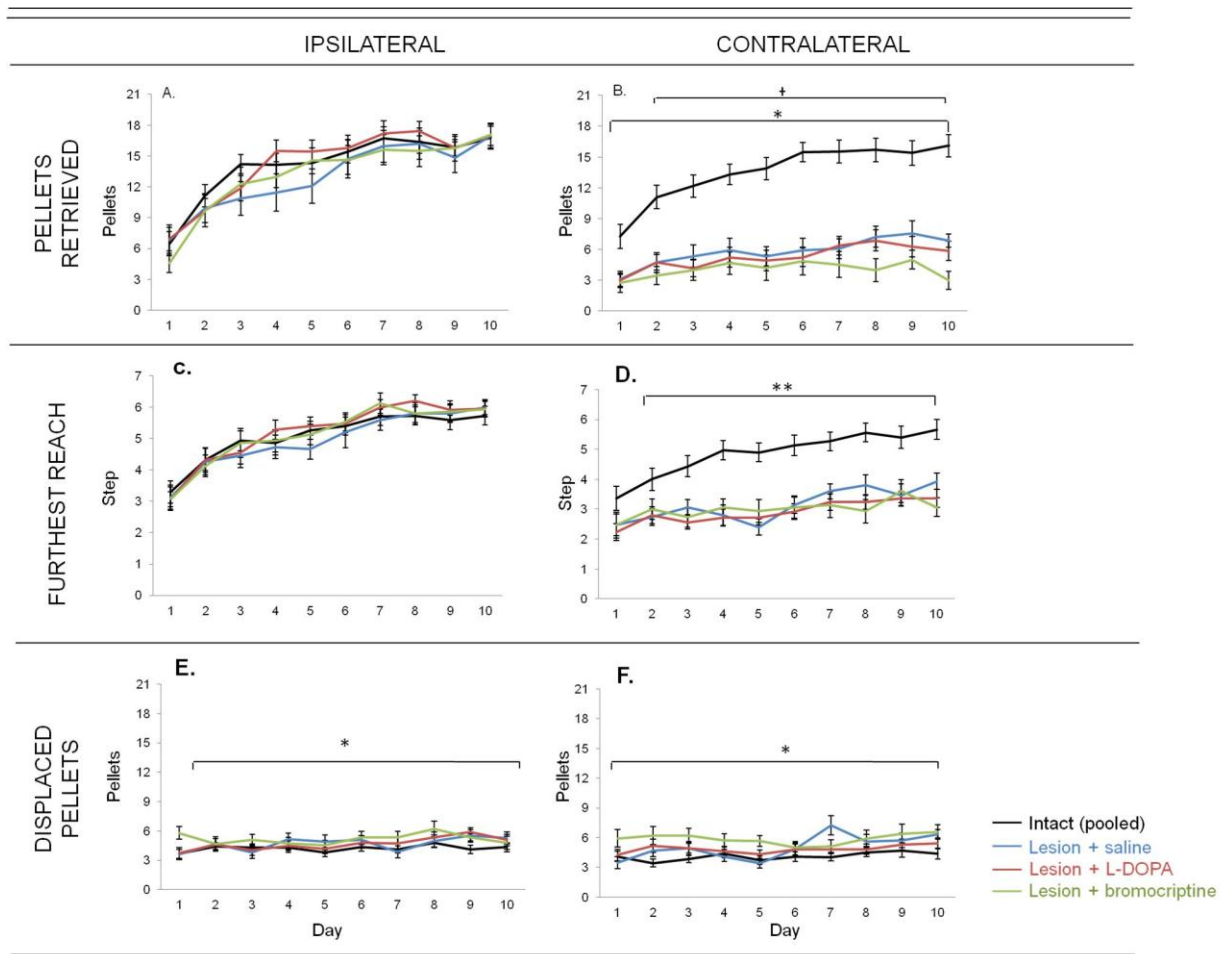


Figure 6.10. The number of pellets obtained from staircase boxes (A-B), the furthest step pellets were retrieved from (C-D), and the number of pellets displaced (E-F) by intact (n=30), lesion drug naïve (n=15), lesion rats chronically treated with L-DOPA (n=25), and lesion rats chronically treated with bromocriptine (n=15) rats. Data from intact rats chronically treated with saline, L-DOPA, or bromocriptine was pooled for clarity.*Intact versus all lesion groups. †Intact versus lesion + bromocriptine. ‡ p<0.05, *p<0.05, **p<0.01.

performance improved over the course of testing regardless of what paw they used, all lesion groups demonstrated a contralateral deficit (Side x Day x Group: $F_{36,720}=1.70$, $p<0.01$). This contralateral impairment was evident from the second day of testing in all lesion rats, apart from those that remained non-dyskinetic following chronic L-DOPA, in whom the deficit manifested itself after the fourth day of testing (Simple Effect of Side: min. $F_{1,80}=4.74$, $p<0.05$). However, there was no overall difference in the performance of the four lesion groups who were all impaired relative to intact controls (Group: $F_{4,80}=4.00$, $p<0.01$).

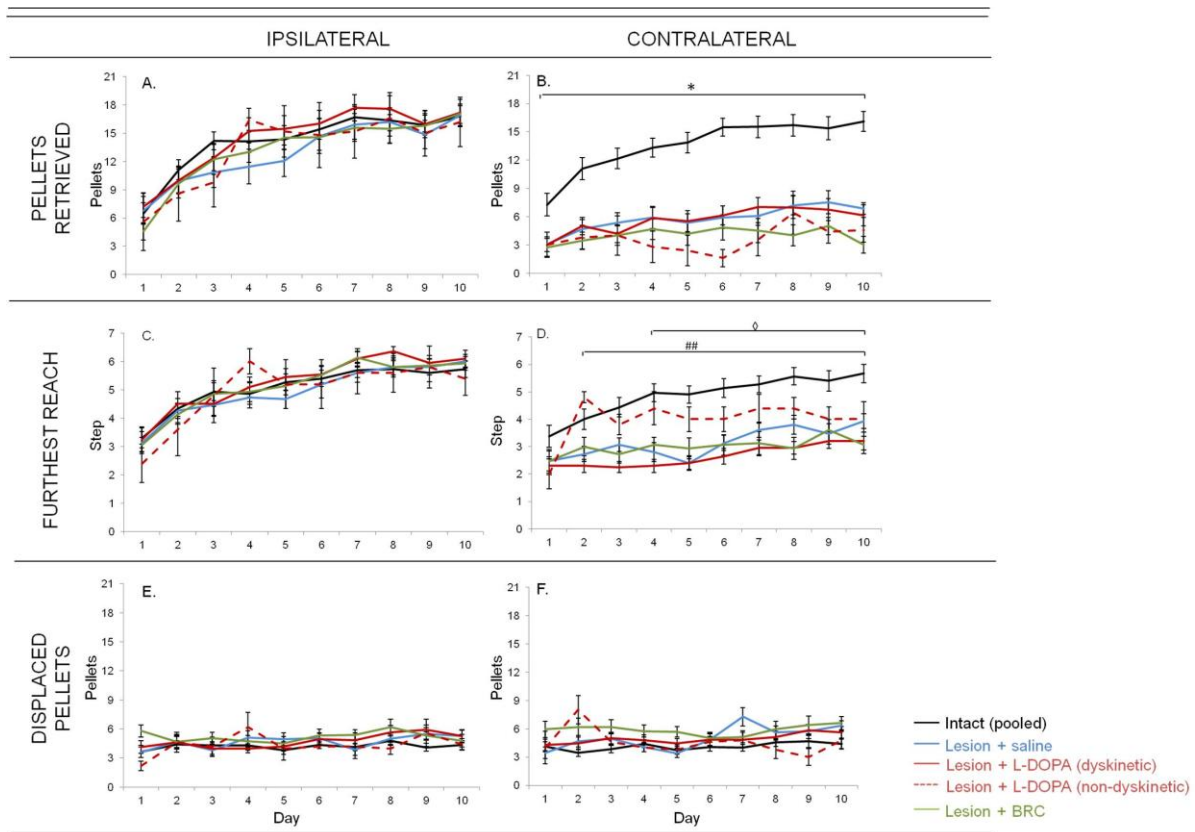


Figure 6.11. The number of pellets obtained from staircase boxes (A-B), the furthest step pellets were retrieved from (C-D), and the number of pellets displaced (E-F) by intact ($n=30$), lesion drug naïve ($n=15$), lesion rats that developed LID following chronic L-DOPA ($n=21$), lesion rat that did not developed LID following chronic L-DOPA ($n=4$), and lesion rats chronically treated with bromocriptine ($n=15$) rats. Data from intact rats chronically treated with saline, L-DOPA, or bromocriptine were pooled for clarity. *Contralateral deficits in all lesion rats, #Contralateral deficit in lesion rats treated with saline, bromocriptine, or rats treated with L-DOPA that developed LID. ◊Contralateral deficit in lesion rats that remained non-dyskinetic following L-DOPA treatment. * $p<0.05$, ◊ $p<0.05$, ## $p<0.01$.

Displaced pellets. Overall, marginally more pellets were displaced by the lesion rats treated with bromocriptine than intact rats (Figure 6.11E-F; Group: $F_{4,80}=3.64$, $p<0.01$). However, there was no difference between the number of pellets that were displaced when intact or lesion rats used their contralateral versus their ipsilateral paw, suggesting that the slight increase in the number of pellets displaced by the lesion bromocriptine treated rats was not due to a contralateral impairment (Side: $F_{1,80}=2.75$, n.s.; Side x Group: $F_{4,80}=0.88$, n.s.). While there were some fluctuations in the number of pellets displaced over the course of testing (Day: $F_{9,720}=3.74$, $p<0.001$), these were similar for intact and lesion rats (Day x

Group: $F_{36,720}=0.89$, n.s.; Side x Day: $F_{9,720}=1.04$, n.s.; Side x Group x Day: $F_{36, 720}=1.36$, n.s.).

6.5. Discussion

The experiments described in this chapter were conducted to test the hypothesis that the disrupted synaptic plasticity associated with LID onset would impair learning of novel motor skills. The hypothesis was based on previously published data suggesting an involvement of the cortico-striatal pathway and synaptic plasticity in the learning of novel motor skills (Luft *et al*, 2004; Yin *et al*, 2009; Wächter *et al*, 2010). Prior to discussing the data, it is, however, noted that the precise role of plasticity in learning has been debated. In addition to direct recordings of e.g. LTP, plasticity may also be assessed by measuring cortical map expansion, i.e. the post-training increase in the size of the cortical areas responding to the trained motor skill. While previous publications have demonstrated that lesions to the nucleus basalis magnocellularis, which disrupt post-lesion cortical map expansion, impair learning of a novel motor skill they do not prevent learning from occurring (Conner *et al*, 2003). This may be taken to suggest that while plasticity enhances learning it is not essential for it to occur, i.e. there is not a direct causal relationship between the two. The potential causal role of plasticity in learning has also been disputed by authors noting that if learning is associated with large scale changes to map plasticity, the learning of a new task should undo previous learning, which is not the case (Kilgard, 2012). Yet other authors have shown that while artificially inducing cortical plasticity in rats outside of a behavioural training context improved subsequent auditory discrimination learning, renormalization of cortical map plasticity 30 days after training did not impact on rats' performance (Rees *et al*, 2011). Hence, while cortical plasticity enhanced learning, renormalization of plasticity did not impact on the learned behaviour (Rees *et al*, 2011).

The sometimes conflicting data on plasticity and learning have led some authors to suggest that the role of cortical map expansion is to increase the pool of neurons, and therefore indirectly the number of neural circuitries, that become activated during performance of a novel task. During learning, it has been suggested that the neural circuitry that is most essential for successful completion of a task is chosen and that, at the end of learning, only this circuitry becomes activated during task performance (Kilgard, 2012). This model may explain why, following cortical map expansion, there is renormalization of the cortical map following completed training (Rees *et al*, 2011) as well as why plasticity is crucial for learning but not maintenance of new skills (e.g. Luft *et al*, 2004).

While the precise role of synaptic plasticity in learning remains a topic of debate, its involvement in the acquisition of novel skills nonetheless led to the hypothesis that chronic L-DOPA treatment to MFB lesion rats, which causes LID in the vast majority of instances (e.g. Winkler *et al*, 2002), would impair learning of a novel motor sequence but not maintenance of a previously acquired motor skill. The hypothesis was tested by measuring rats' performance in staircase boxes. In line with previous experiments (Montoya *et al*, 1995; Wishaw *et al*, 1997) unilateral lesions decreased the number of pellets that rats were able to retrieve using their contralateral paw. In accordance with the hypothesis, Experiment 6 demonstrated that L-DOPA treatment did not affect performance of lesion rats that were pre-trained on the task before lesion and drug treatment.

Experiments 7 and 8 tested the second part of the hypothesis, i.e. the effect of dopamine denervation and exposure to chronic L-DOPA on rats' ability to learn how to successfully retrieve sucrose pellets from staircase boxes *de novo*. Data from Experiment 7 suggested that chronic L-DOPA treatment may induce a mild impairment in the rate with which rats that had not received staircase training prior to lesion learned to perform the task. This interpretation was based on the diminished ability of lesion L-DOPA treated rats to collect pellets located on the steps located further away, i.e. the pellets that were more difficult for rats to retrieve, during initial testing days. Interestingly there was also a trend towards a decrease in the number of ipsilateral pellets they retrieved (Figure 6.6A-C). The bilateral nature of the deficit was unexpected but may be explained by data suggesting that, despite being designed as a lateralized motor test, successful performance on the staircase test requires coordination of both ipsilateral and contralateral muscles (Wishaw *et al*, 1997). It is also recognised that some authors have demonstrated bilateral changes in monoamine neurochemistry following unilateral lesion lesions (Berger *et al*, 1991; Pierucci *et al*, 2009), and that this may have impacted on ipsilateral performance. Therefore, while the lesion may have created a flooring effect when measuring performance on the more impaired contralateral side, an effect of chronic L-DOPA on lesion rats' learning may have been more readily observed on the ipsilateral side where rats were less impaired and where there was therefore no flooring effect (Wishaw *et al*, 1997). Nonetheless, when discussing the data from Experiment 7 it is caveated that the observed difference between lesion rats treated with saline and L-DOPA was mild and only apparent in the beginning of the ten day testing period. It was therefore considered necessary to replicate the effect before any conclusions on the effect of chronic L-DOPA on staircase learning could be drawn.

In addition it was noted that a difference in performance of lesion rats treated with saline or chronic L-DOPA may have related to non-motor factors such as motivation rather than ability for motor skill learning. Therefore, the time taken for the groups to consume 100 freely available pellets was compared at the end of staircase testing in Experiment 7 to provide a gross estimate of motivation to consume sucrose pellets. Whilst there is a motor component to the sucrose consumption task, which likely explains differences between the intact and lesion groups, there were no significant differences in the two lesion groups' performance. This suggests that the two lesion groups did not differ in their motivation to consume freely available sucrose pellets, and that the effect of chronic L-DOPA on learning in staircase boxes was unlikely related to crude motivational factors.

Having identified a potential effect of chronic L-DOPA on lesion rats' learning on the staircase task, a further experiment was conducted to replicate the findings. As it had been hypothesised that an effect of chronic L-DOPA on learning would be linked to the disrupted synaptic plasticity that is associated with the onset of LID, this later experiment also included a lesion group that was chronically treated with the D2 agonist bromocriptine following lesion. Unlike L-DOPA, bromocriptine does not induce LID in lesion rats (Pearce *et al*, 1998) and was therefore hypothesised to not affect learning. As the difference between the lesion saline and lesion L-DOPA group in Experiment 7 had been minimal, an attempt was also made to increase the sensitivity of the test, and therefore the likelihood of observing a difference between the experimental groups, by increasing the number of pellets available on each step from three to four. As hypothesised; lesion rats chronically treated with bromocriptine did not differ from lesion saline treated controls in their acquisition of the staircase task. This suggests that treatment with the clinically available D2 agonist bromocriptine does not impair acquisition of a novel motor skill in 6-OHDA lesion rats as measured using the staircase task.

However, contrary to previous findings, no deficit following L-DOPA treatment of lesion rats was found in section 6.3. It is noted that the effect of chronic L-DOPA on lesion rats' synaptic plasticity differs depending on whether or not rats develop LID. While all lesion rats in Experiment 7 developed LID in response to chronic L-DOPA, a subgroup of L-DOPA treated lesion rats in Experiment 8 remained non-dyskinetic. To exclude the possibility that the different results was due to the proportion of lesion L-DOPA treated rats that developed LID, a further analysis of the data in Experiment 8 was conducted wherein the lesion L-DOPA treated group was split into a dyskinetic and non-dyskinetic cohort. This second analysis confirmed that the effect observed in dyskinetic rats in Experiment 7 could

not be replicated in Experiment 8. While there were some behavioural differences between L-DOPA treated rats that did or did not develop LID in Experiment 8, these were not very pronounced (Figure 6.11). Furthermore, considering the different effects of LID onset in Experiments 7 and 8, any statistical difference between dyskinetic and non-dyskinetic rats should be treated with caution as the behaviour in the dyskinetic control group is not replicable.

The inability to replicate the findings may have been due to differences in the methodology of the Experiments 7 and 8. Experiment 7 included a more extended food restriction prior to staircase testing than Experiment 8 (total of 70 vs. 44 days). Food restriction is known to increase the sensitivity of D1 and D2 receptors, both of which are implied in striatal plasticity, (Carr *et al*, 2003) as well as to affect motor cortex plasticity by enhancing LTD induction (Cohen & Castro-Alamancos, 2005). The possibility that the extended food restriction, which was a prerequisite for the operant training in Experiment 8, may have impacted on dopamine receptors and synaptic plasticity in those rats cannot be excluded.

Considering the different findings in Experiments 7 and 8, and the possibility that food restriction may be hypothesised to affect staircase learning by impacting on synaptic plasticity (Carr *et al*, 2003; Cohen & Castro-Alamancos, 2005), future studies may wish to further test the effect of chronic L-DOPA treatment utilizing behavioural tasks that do not require food restriction. One such task is the rotarod task. Learning on the rotarod task has previously been linked to changes in synaptic plasticity, characterised by higher synaptic strength in the dorsomedial striatum during early training phases but higher synaptic strength in the dorsolateral striatum after extended training (Yin *et al*, 2009). As such, the rotarod task may be a suitable paradigm for testing the hypothesis that LID onset impairs motor learning. Unfortunately, due to limited availability to rat rotarods this paradigm was not used in this project.

In all, the data presented in this chapter support the hypothesis that L-DOPA treatment does not affect lesion rats' ability to perform motor skills learned prior to lesion and L-DOPA treatment. However, they did not provide replicable support for the hypothesis that chronic L-DOPA treatment impairs lesion rats' ability to learn *de novo* motor skills as measured using the staircase task. Future experiments may wish to test whether an effect can be found if using motor learning paradigms that do not require food restriction.

7. Dopaminergic Treatments & the Lateralised Choice Reaction Time Task

Background: The LCRT task can measure attention (reaction time), motor (reaction time and movement time) and non-motor (accuracy of responding) function in the unilateral rat 6-OHDA model of PD. Previous authors have demonstrated that rats' performance on the LCRT task is mediated by the striatal dopaminergic system and that both motor and non-motor performance on the task is disrupted by unilateral 6-OHDA lesions.

Aims: The aim of the experiments presented in this chapter was to test the effect of acute and chronic dopaminergic treatments on lesion rats' motor and non-motor performance on the LCRT task in MFB lesion rats.

Methods: The effects of acute and chronic bromocriptine and L-DOPA in intact rats and MFB lesion rats were explored in three separate experiments, all using the LCRT task. The first experiment tested the effects of acute bromocriptine administration on lesion rats' LCRT task performance. The second experiment was designed to (i) replicate findings of a long-lasting effect of acute bromocriptine on the accuracy of contralateral responding, and (ii) test the effect of chronic L-DOPA administration on later LCRT task performance in lesion rats. The third experiment was designed to replicate previous findings of an effect of chronic L-DOPA treatment on LCRT task performance, and compare it with the effects of chronic bromocriptine administration. In addition, the experiment tested the effect of acute L-DOPA and bromocriptine on LCRT task performance.

Results: 6-OHDA lesions impaired both motor and non-motor performance on the LCRT task. Acute bromocriptine improved lesion rats' accuracy of responding but had limited effect on motor function. The reverse trend was observed following acute L-DOPA administration, which improved motor function but did not affect accuracy of responding. Chronic L-DOPA, but not chronic bromocriptine, caused a further impairment of lesion rats' non-motor function.

7.1. Introduction

The previous chapter described experiments designed to the effect of chronic L-DOPA treatment on lesion rats' acquisition of a novel motor skill. However, in addition to motor learning (e.g. Wächter *et al*, 2010), dopamine input to the striatum is also implicated in non-motor functions such as reward signalling, and goal directed and habitual behaviour (Schultz 2010; Yin *et al*, 2005; Faure *et al*, 2005). Chronic L-DOPA induces changes to the striatal system in the form of sensitised dopamine receptors (e.g. Aubert *et al*, 2005; Konradi *et al*, 2004) and altered plasticity (e.g. Picconi *et al*, 2003). In addition to causing LID, it is therefore likely that chronic L-DOPA could affect performance on operant paradigms measuring goal directed and/or habitual behaviour reliant on striatal function.

LID can be induced in lesion rats by administering them chronic L-DOPA. While LID may be induced in both rats with intra-striatal and MFB lesions, it is more consistently induced in the latter model (Winkler *et al*, 2002). It is also in the unilateral MFB lesion model that previous authors have demonstrated an effect of LID onset of cortico-striatal plasticity (Picconi *et al*, 2002). For these reason, the unilateral 6-OHDA, MFB model was utilised in the experiments presented in this chapter.

This choice of lesion model required an operant paradigm that did not only tap into striatally mediated behaviour, but that was also designed for use in rats with unilateral lesions and that could measured both motor and non-motor behaviour. The current chapter therefore used the LCRT task, which is described in detail in sections 1.7.6.1 and 2.7.2. Briefly, the task takes place in nine-hole boxes where rats are required to respond to the onset of a central light cue by nose poking into the hole the light was presented in. After a variable delay, a lateralised light cue is presented for 50 ms to either the contralateral or ipsilateral side of the lesion, and rats are rewarded with a sucrose pellet if discontinuing the central hold and nose poking into the recently illuminated hole. The LCRT task provides measurements of the number of trials rats initiate, their reaction time, movement time, and the accuracy with which they respond to lateralised cues. Of these parameters, the movement time has the strongest motor element to it. While reaction time is influenced by motor function, it also has an attention aspect to it as an effect of lesion on reaction but not movement times would indicate an attention rather than motor deficit. Conversely, while accurate responding requires a motor response, continued accurate responding also requires maintenance of stimulus-response association and unlike the previous measurements this parameter therefore also measures non-motor function.

In addition to impairing reaction and movement times, unilateral 6-OHDA lesions have been shown to induce a gradual decline in the accuracy of rats' contralateral responding (Dowd & Dunnett, 2004, 2005a,b). While the nature of this deficit has not been conclusively determined, it has been hypothesised that 6-OHDA lesions cause loss of the dopaminergic reward signal which otherwise occurs following presentation of a CS (Dowd & Dunnett, 2004; Schultz, 2010). This, in turn has been hypothesised to lead to extinction of contralateral responding, reflected in the gradual decrease in lesion rats' contralateral accuracy (Dowd & Dunnett, 2004, 2007).

Three experiments, described in sections 7.2, 7.3, and 7.4, were conducted. In section 7.2, the effect of acute administration of a D2 agonist on LCRT task performance was tested; section 7.3 describes an experiment designed to replicate the first experiment while also assessing the effect of chronic L-DOPA on LCRT task performance; and section 7.4 describes an experiment testing the effect of chronic and acute L-DOPA and bromocriptine treatment on LCRT task performance.

7.2. Experiment 9: The effect of D2 agonism on LCRT task performance

7.2.1. Introduction

While previous publications have demonstrated that accurate performance on the LCRT task relies on striatal dopamine (Carli *et al.*, 1989; Dowd & Dunnett, 2004), no studies investigating the underlying pharmacology have been reported. In addition to replicating the previously reported gradual decline in lesion rats' contralateral accuracy on the LCRT task (Dowd & Dunnett, 2004, 2005a,b), this experiment therefore also sought to test the effect of acute agonism of D2 receptors on lesion rats' LCRT task performance.

This was accomplished using bromocriptine, which primarily binds to D2 receptors but also has affinity for D3 receptors as well as minimal binding to other dopaminergic and serotonergic receptors (Table 7.1). Bromocriptine alleviates motor deficits associated with PD, and has been used in the clinic since the 1970's (Lieberman & Goldstein, 1985). In addition to motor symptoms, previous publications also suggest a role of D2 receptors in non-motor behaviour. For example, the effect of D2 antagonism on a reaction time paradigm has previously been tested by Smith and colleagues (2000). Their paradigm took place in two-lever operant boxes where rats were required to sustain a lever press for a variable length of time until the onset of a visual light cue (CS). Releasing the lever within 700 ms of CS presentation resulted in the delivery of a food pellet. Smith and colleagues (2000)

demonstrated that antagonism of D2, but not D1 or D3, receptors decreased the number of trials in which rats successfully released the lever press within the 700 ms period. Because the number of trials the rats completed was similar regardless of the time (300, 500, 700, or 900 ms) which rats had to sustain the lever press for before presentation of a CS, it was argued that the decrease in responding was not reflective of rats merely always pressing the lever for the same length of time; and the possibility that the decreased responding could reflect a decrease of reward efficacy of the sucrose pellet was presented (Smith *et al*, 2000). Other authors have shown that agonism of D2 receptors by acute bromocriptine increase the frequency with which rats press a reinforced, relative to a non-reinforced, lever in order to obtain a conditioned reward (Beninger & Ranaldi, 1992; Ranaldi & Beninger, 1995; Sutton *et al*, 2001). Based on the previously published hypothesis that 6-OHDA lesions decrease accuracy in the LCRT task because of a lesion-induced loss of the dopamine reward signal rather than loss of motor function (Dowd & Dunnett, 2004, 2007), and the ability of bromocriptine to not only mimic the effect of the dopamine that is lost following 6-OHDA lesions, but also enhance responding for a reward (Beninger & Ranaldi, 1992; Ranaldi & Beninger, 1995; Sutton *et al*, 2001), it was hypothesized that acute bromocriptine administration would increase lesion rats' accuracy on the day of administration. This hypothesis was tested over a 10 day testing cycle in which bromocriptine was administered to lesion rats on the second day of testing.

In a final testing cycle, the same rats were administered acute raclopride prior to operant testing. Raclopride is a dopamine antagonist with high affinity for D2 receptors, as detailed in Table 7.2. Because raclopride primarily inhibits D2 receptors (Köhler *et al*, 1985) it was hypothesised that acute administration of the antagonist would have the opposite effect to acute bromocriptine on LCRT task performance. Hence, raclopride was used to control whether an effect of bromocriptine on accuracy was caused by the acute agonism of, primarily, D2 receptors. Furthermore, based on previous publications showing that raclopride worsens reaction and movement times in intact Sprague Dawley rats performing a reaction time task (MacDonald *et al*, 2006), it was hypothesized that acute raclopride would induce a transient reaction and movement time deficit in intact rats, as well as further impairing the reaction and movement time deficit observed in lesion rats.

Table 7.1. Bromocriptine's binding affinities (K_i in nmol/L) for dopaminergic and serotonergic receptors. Adapted from Kvernmo *et al* (2006).

| D1type | D2type | D ₂ | D ₃ | D ₄ | D ₅ | 5-HT _{1B} | 5-HT _{1B} | 5-HT _{1D} | 5-HT _{2A} | 5-HT _{2B} | 5-HT _{2C} |
|--------|--------|----------------|----------------|----------------|----------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| 1659 | 12.2 | 2.5 | 12.2 | 59.7 | 1691 | 12.9 | 354.8 | 10.7 | 107.2 | 56.2 | 741.3 |

Table 7.2. Raclopride's inhibitory potency (IC_{50} , μ M) on receptors in the rat striatum. Adapted from Köhler *et al* (1985).

| D1 | D2 | D3 | α_1 - adrenergic | α_2 - adrenergic | β - adrenergic | 5HT ₁ | 5HT ₂ | Histamine H ₁ | Muscarinic |
|------|-------|------|----------------------------|----------------------------|-------------------------|------------------|------------------|-----------------------------|------------|
| >100 | 0.026 | 0.90 | 32 | 38 | >100 | 49 | 13 | 8.4 | >100 |

7.2.1.1. Aims

The aim of the experiment was to (i) replicate previous findings of a gradual decline in contralateral accuracy following 6-OHDA lesions, and (ii) probe the neurobiological mechanisms underlying the contralateral accuracy deficit on the LCRT task by testing the effect of acute administration of the D2 receptor agonist, bromocriptine, on LCRT task performance.

7.2.2. Methods

7.2.2.1. Experimental design

All rats were pre-trained on the LCRT task before a subgroup was given unilateral 6-OHDA lesions to the MFB. Following recovery, rats were tested on the LCRT task at 3 and 8 weeks post-lesion. At the end of the second testing cycle, all lesion rats were administered acute bromocriptine prior to operant testing (Figure 7.1).

The original experimental design planned for the lesion group to be tested on the LCRT task following acute administration of three doses of bromocriptine, with a wash out period in between each. The intention was then to select the most effective dose and compare the performance of lesion rats tested on and off bromocriptine at this dose. Due to unforeseen effects of bromocriptine administration, the design was altered and rats were tested on the LCRT task off-medication for 9 days following one initial bromocriptine administration. Performance on the LCRT task, the vibrissae test, and the adjusting step test was thereafter assessed following acute administration of the D2 antagonist raclopride.

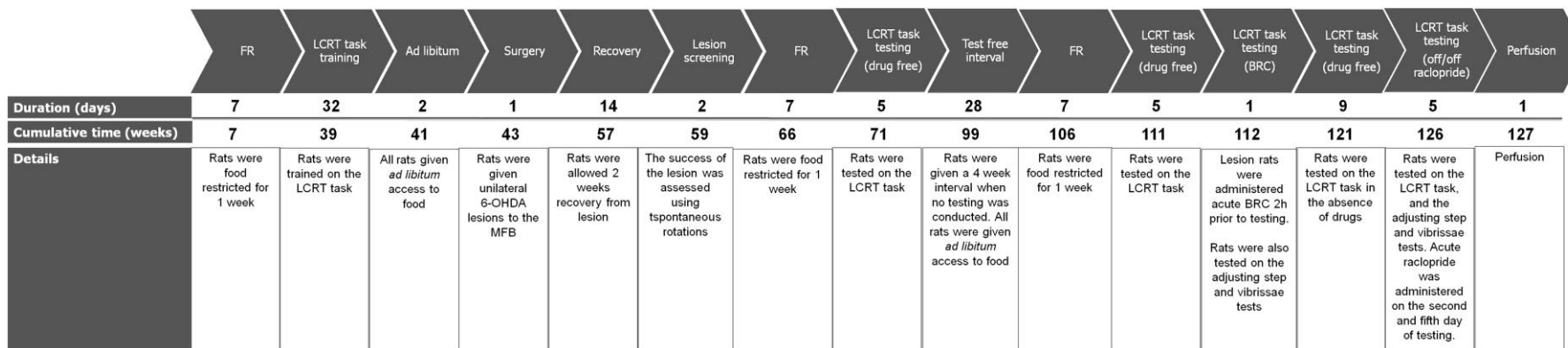


Figure 7.1. A graphical representation of the experimental design of Experiment 9. The figure specifies both the duration of the individual testing phases, and the overall number of days in the experiment. FR=Food restriction; BRC=bromocriptine; LCRT task=Lateralised choice reaction time task; MFB=Medial forebrain bundle.

7.2.2.2. Procedure

The gradual decrease in lesion rats' contralateral accuracy on the LCRT task reported by Dowd & Dunnett (2004, 2005a,b) had been observed using male rats. To replicate the original findings as closely as possible, the first experiment in the chapter therefore included male rats.

Twenty-two male Lister Hooded rats received 32 days of training on the LCRT task (section 2.7.2.2) after which a cohort of 12 rats were given unilateral 6-OHDA lesions to the MFB (section 2.2.2). Two weeks post-lesion, lesion extent was assessed using spontaneous rotations (section 2.4.6.3). The intact (n=10) and lesion (n=12) groups' performance on the operant task was then tested for five consecutive days, at 3 and at 8 weeks post-lesion. Immediately following the second post-lesion testing cycle, rats were retested on the LCRT task and the lesion group received 1.25 mg/kg bromocriptine, prepared and administered as described in section 2.3.1, two hours prior to operant testing. Both groups were thereafter tested in the absence of any drug for a further nine consecutive days. Finally, the effect of raclopride

on LCRT task and motor performance was assessed in a three day testing cycle where 0.2 mg/kg raclopride (dose chosen based on in-house data), prepared as described in section 2.3.5, was administered 30 min prior to testing on the second day. Motor performance was measured the day before, of, and after raclopride administration using the adjusting step (section 2.4.3) and vibrissae (section 2.4.8) tests. Motor tests were always conducted immediately following operant testing. These tests were chosen because of their non-spontaneous nature which, unlike e.g. the cylinder test, guaranteed that a motor response could be recorded within a limited amount of time. In addition, two more days of testing were conducted to determine the effect of 0.4 mg/kg raclopride on motor function and LCRT task performance. However, at this dose the antagonist suppressed motor function to the extent that no LCRT task data could be gathered and this data is as such not reported.

At the end of the experiment, rats were administered an overdose of Euthatal and perfused (section 2.8). The tissue was used for TH immunohistological staining (section 2.10). Dopaminergic cell loss in the SNc and VTA was assessed by counting TH+ cell bodies (section 2.11) whereas dopamine denervation in the striatum was assessed by measuring optical density (section 2.12).

7.2.2.3. Statistical analysis

Dopamine denervation was assessed using a paired samples t-test. LCRT task performance 3 and 8 weeks post-lesion was analysed using a repeated measures ANOVA with Group as between-subject factor and Cycle, Side, and Day as within-subject factors. The effect of acute bromocriptine was measured using a repeated measures ANOVA with Group as between-subject factor and Day as within-subject factor. Days included the day before (baseline), the day of drug administration, and the nine days after post-bromocriptine exposure. The effect of raclopride on the vibrissae and adjusting step tests and was analysed using a repeated measures ANOVA with Group as a between-subject factor and Day and Side as within-subject factors. Significant between-subject effects were analysed using a post-hoc Scheffe test and significant interactions analysed using a test of simple effects.

7.2.3. Results

7.2.3.1. Lesion effect

The lesion caused a profound loss of dopaminergic cell bodies that was more pronounced in the SNc than in the VTA (Figure 7.2; $t_{11}=3.48$, $p<0.01$). In addition, there was a significant reduction of striatal terminals as assessed by comparing optical density in the ipsilateral and contralateral striatum (percentage of intact side: $31\% \pm 3\%$; $t_{11}=13.82$, $p<0.01$).

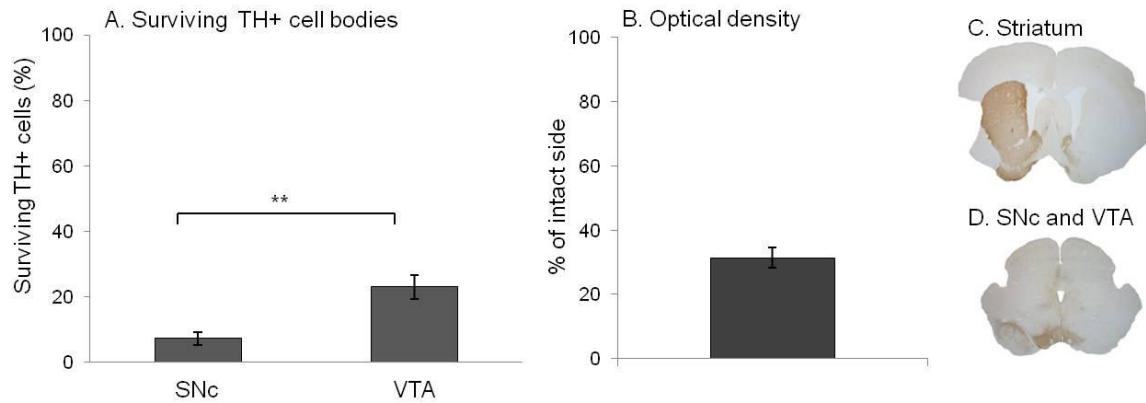


Figure 7.2. The bar charts show the mean surviving dopaminergic cell bodies in the lesion SNc and VTA (A), and the optical density (B) in their lesion striatum of the lesion group (n=12). Both are shown as a percentage of the intact side. The photos depict the loss of dopaminergic cell bodies in the striatum (C), SNc, and the VTA (D). The error bars represent the standard error of the mean. SNc=Substantia nigra pars compacta; TH=Tyrosine hydroxylase; VTA=Ventral tegmental area. **p<0.01

7.2.3.2. Effect of DA depletion on LCRT task performance

Usable trials. Lesion rats made fewer usable trials than intact controls (Figure 7.3A; Group: $F_{1,20}=43.38$, $p<0.001$). The lesion group showed a small but significant decrease in their number of usable trials over the course of testing (Day x Group: $F_{4,80}=13.32$, $p<0.001$; Simple Effect: min. $F_{4,17}=3.75$, $p<0.05$; Day: $F_{4,80}=0.32$, n.s.; Cycle x Day: $F_{4,80}=1.43$, n.s.; Cycle x Day x Group: $F_{4,80}=0.30$, n.s.). The number of usable trials made by the rats was similar when tested three and eight weeks post-lesion (Cycle: $F_{1,20}=0.99$, n.s.; Cycle x Group: $F_{1,20}=4.15$, n.s.).

Reaction time. Overall, the lesion group had significantly longer reaction times than intact controls (Figure 7.3B-C; Group: $F_{1,20}=167.83$, $p<0.001$). While, at three weeks post-lesion, the lesion group showed longer reaction times than controls following both presentation of ipsilateral and contralateral cues, only the contralateral deficit remained significant at eight weeks post-lesion (Cycle x Side x Group: $F_{1,20}=5.93$, $p<0.05$; Simple Effect: min. $F_{1,20}=4.37$, $p<0.05$). The lesion rats' contralateral reaction time deficit gradually worsened over the five-day testing cycle (Side x Day x Group: $F_{4,80}=13.18$, $p<0.001$; Simple Effect: min. $F_{4,17}=3.8$, $p<0.05$). As a result of the gradual nature of the deficit, the lesion rats' contralateral reaction time was similar to that of controls on the first day of each testing cycle but significantly longer the remaining four days of each cycle (min. $F_{1,20}=30.29$, $p<0.001$).

Movement Time. Lesion rats' movement times were longer than those of intact controls. The deficit manifested itself when rats made contralateral responses, and gradually worsened over the course of each five-day testing cycle (Figure 7.3D-E; Group: $F_{1,20}=54.88$, $p<0.001$; Side x Day x Group: $F_{4,80}=2.64$, $p<0.05$; Simple Effect: $F_{4,17}=5.10$, $p<0.01$). Overall, the contralateral deficit was greater eight than three weeks post lesion (Cycle x Side x Group: $F_{1,20}=7.51$, $p<0.05$; Simple Effect: $F_{1,20}=28.65$, $p<0.001$).

Accuracy. The lesion rats were significantly less accurate in their responding than intact controls (Figure 7.3F-G; Group: $F_{1,20}=52.92$, $p<0.001$) both three and eight weeks post-lesion (Cycle: $F_{1,20}=0.44$, n.s.; Cycle x Group: $F_{1,20}=0.50$, n.s.). In line with previous publications, there was a gradual decline in the accuracy with which lesion rats responded to contralateral cues (Side x Day x Group: $F_{4,80}=3.91$, $p<0.01$; Simple Effect: $F_{4,17}=17.03$, $p<0.001$).

7.2.3.3. Effect of acute bromocriptine on LCRT task performance

The effect of acute bromocriptine on LCRT task performance was measured in an 11 day cycle, where bromocriptine was administered to lesion rats on the second day of testing.

Usable trials. The lesion group continued making fewer usable trials than intact controls during the bromocriptine testing cycle (Figure 7.4A; Group: $F_{1,20}=41.29$, $p<0.001$). There were some fluctuations in the number of usable trials made by the lesion but not intact rats (Day x Group: $F_{10,200}=6.75$, $p<0.001$; Simple Effect: $F_{9,12}=3.00$, $p<0.05$) but these were not specific to the day of bromocriptine administration.

Reaction time. The lesion group continued showing longer reaction times than intact controls when responding to cues presented on their contralateral side (Figure 7.4B-C; Group: $F_{1,20}=8.08$, $p<0.05$; Group x Side: $F_{1,20}=50.18$, $p<0.001$; Simple Effect: $F_{1,20}=114.80$, $p<0.001$). Reaction time did not decrease on the day of acute bromocriptine administration (Day: $F_{10,200}=1.14$, n.s.; Day x Group: $F_{10,200}=1.07$, n.s.; Side x Day: $F_{10,200}=1.23$, n.s.; Side x Day x Group: $F_{10,200}=1.48$, n.s.).

Movement time. The lesion group continued showing longer movement time than intact controls when responding to contralateral cues throughout the testing cycle (Figure 7.3D-E; Group x Side: $F_{10,200}=3.52$, $p<0.001$; Simple Effect: $\min.F_{1,20}=10.44$, $p<0.01$).

Accuracy. Lesion rats continued being less accurate than intact controls when required to respond to contralateral cues (Figure 7.3F-G; Group: $F_{1,20}=16.86$, $p<0.01$; Side x Group: $F_{1,20}=7.30$, $p<0.05$; Simple Effect: $F_{1,20}=16.92$, $p<0.01$). As hypothesised, acute bromocriptine administration increased the lesion group's accuracy on the day of

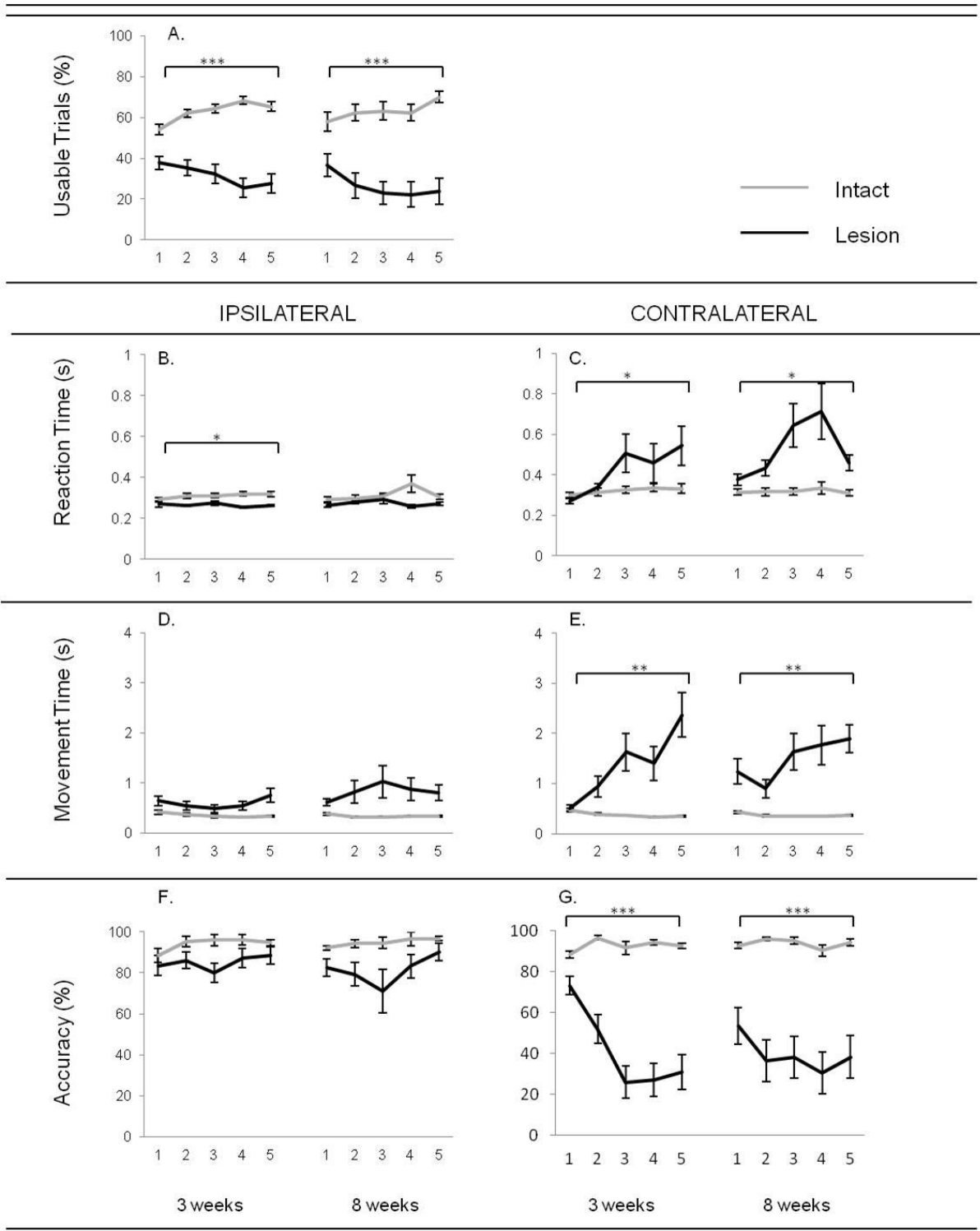


Figure 7.3. The intact (n=10) and lesion (n=12) groups' usable trials (A), together with their ipsilateral and contralateral correct reaction time (B-C), correct movement time (D-E), and accuracy (F-G) when tested 3 and 8 weeks post-lesion. The error bars represent the standard error of the mean. *p<0.05, **p<0.01, ***p<0.001

administration. Interestingly, while this increase in accuracy was expected to be transient, the contralateral accuracy of the lesion group did not return to baseline the day after bromocriptine administration. Therefore, testing continued for nine days post bromocriptine injection. Lesion rats continued being significantly less accurate than controls the day of, and four days after acute bromocriptine administration. However, because of a gradual increase in contralateral responding, beginning on the day of acute bromocriptine administration, lesion rats' performance was similar to that of intact controls five days after acute bromocriptine after which it stabilised (Day x Group: $F_{10,200}=2.11$, $p<0.05$; Simple Effect: min. $F_{1,20}=6.97$, $p<0.05$).

7.2.3.4. Effect of acute raclopride on simple motor and LCRT task performance

The effect of 0.2 mg/kg acute raclopride on behaviour was assessed in a three day cycle using the vibrissae test, the adjusting step test, and the LCRT task. Unlike the long-lasting effect of bromocriptine, the effect of raclopride was transient and only observed on the day of administration.

Adjusting Step Test. As expected, the lesion group made overall fewer adjusting steps than intact controls (Figure 7.5A-B; Group: $F_{1,20}=39.75$, $p<0.001$). Acute raclopride administration decreased the number of ipsilateral and contralateral adjusting steps made by both groups (Side x Day x Group: $F_{2,40}=22.93$, $p<0.001$; Simple Effect: min. $F_{2,19}=18.15$, $p<0.001$).

Vibrissae test. The lesion group made fewer paw placements in response to vibrissae stimulation than intact controls (Figure 7.5C-D; Group: $F_{1,20}=71.29$, $p<0.001$). Acute raclopride administration decreased both ipsilateral and contralateral motor performance in the intact group whereas, due to their pre-existing contralateral impairment, it only further decreased the number of ipsilateral responses made in the lesion group (Side x Day x Group: $F_{2,40}=28.06$, $p<0.001$; Simple Effect: $F_{2,19}=41.58$, $p<0.001$).

Usable trials. While the lesion group continued making overall fewer usable trials than intact controls (Figure 7.6A; Group: $F_{1,20}=13.58$, $p<0.01$) the intact group's usable trials decreased to the same level as the lesion group on the day of acute raclopride administration (Day x Group: $F_{2,40}=8.04$, $p<0.01$; Simple Effect on days 1 and 3: min. $F_{1,20}=30.59$, $p<0.001$).

Reaction time. The lesion group continued showing longer reaction times than intact controls (Figure 7.6B-C; Group: $F_{1,20}=6.19$, $p<0.05$; Side x Group: $F_{1,20}=19.71$, $p<0.001$; Simple Effect: $F_{1,20}=49.47$, $p<0.001$). Neither lesion nor intact rats' reaction time increased

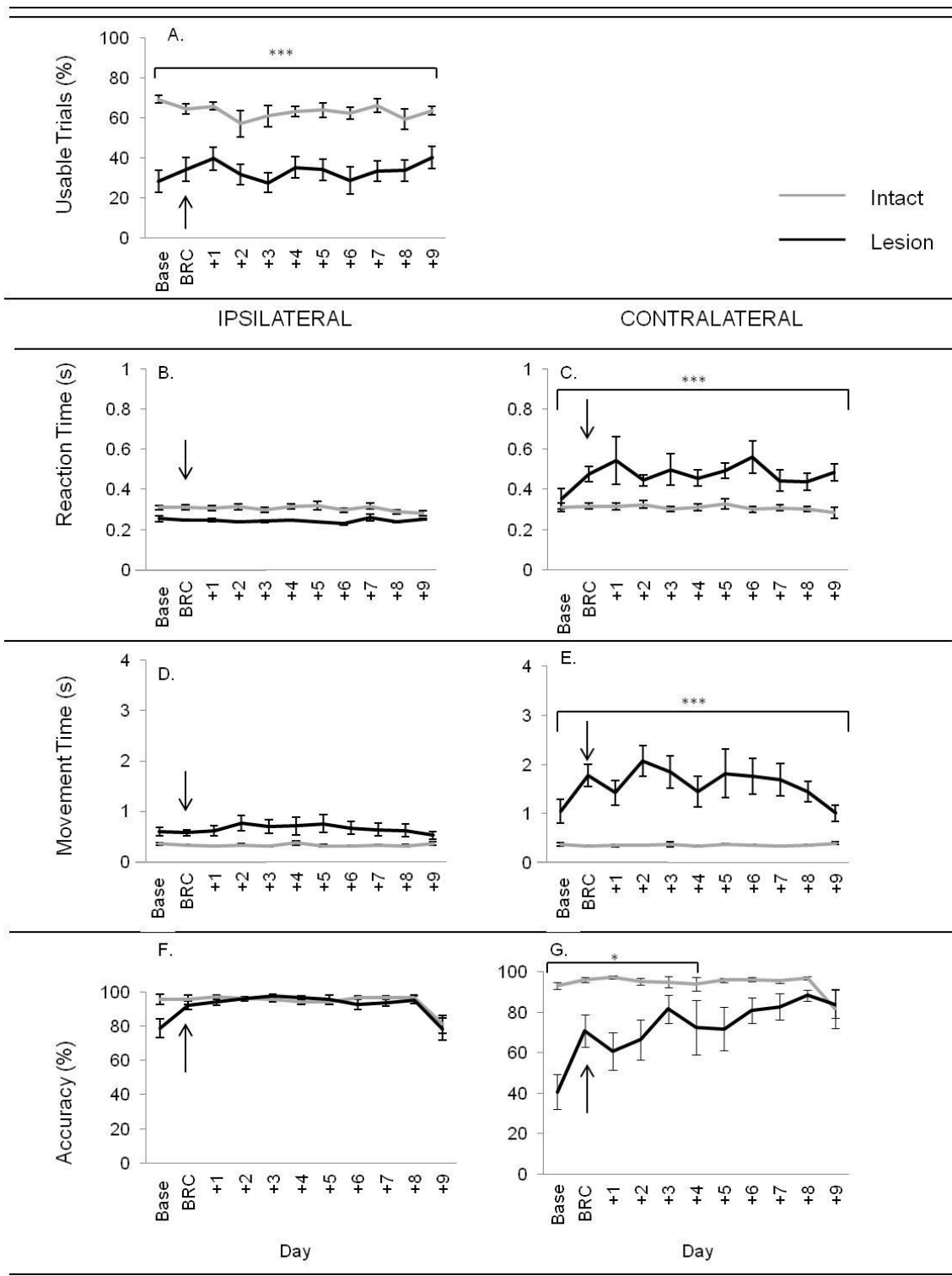


Figure 7.4. The usable trials (A) and ipsilateral and contralateral reaction time (B-C), movement time (D-E), and accuracy (F-G) of intact (n=10) and lesion (n=12) rats before and after administration of acute bromocriptine. The arrows represent the day of bromocriptine administration. The line chart show the mean performance and the error bars represent the standard error of the mean. Base=baseline, BRC=bromocriptine. *p<0.05, **p<0.01, *p<0.001**

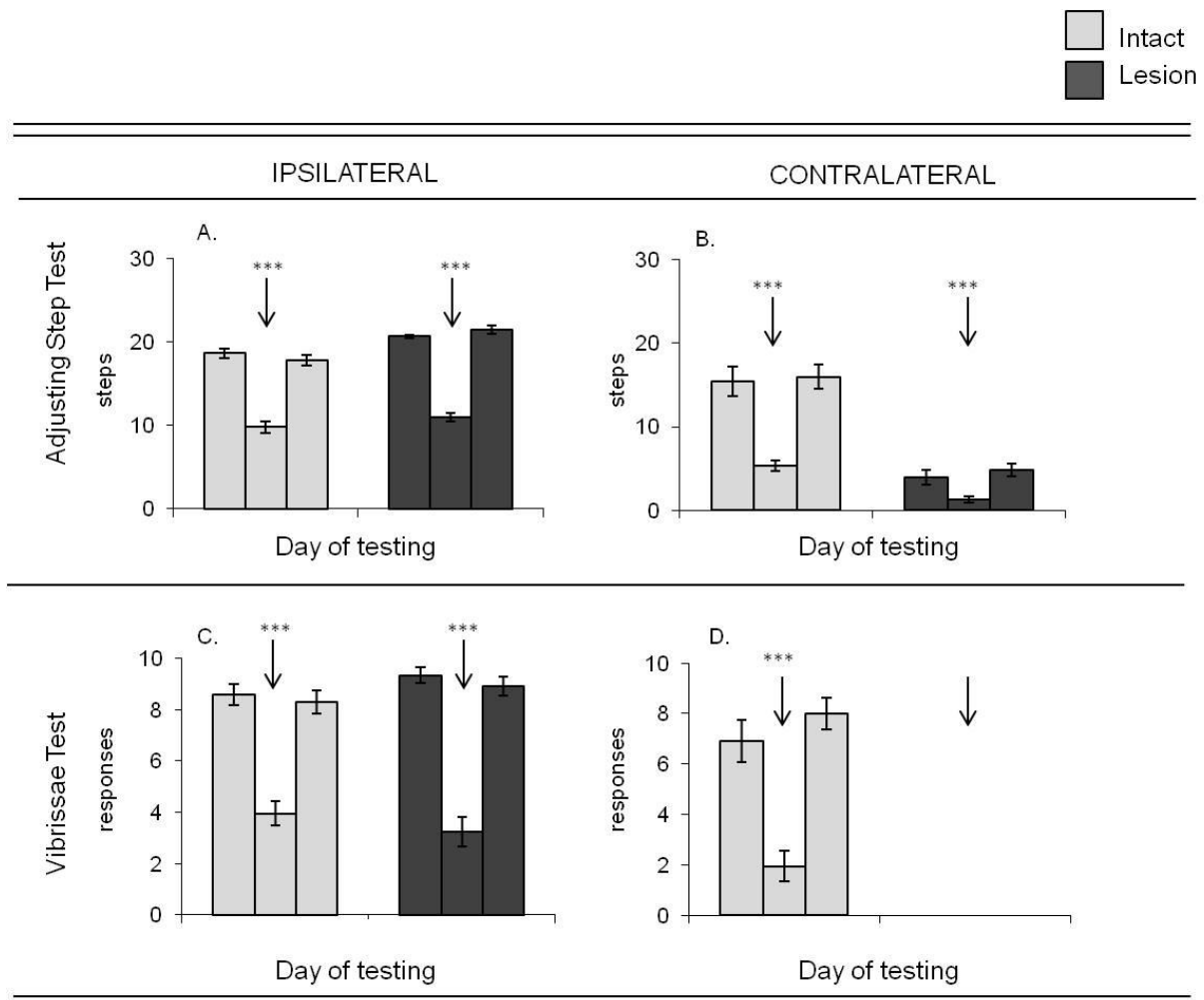


Figure 7.5. The ipsilateral and contralateral performance of intact (n=10) and lesion (n=12) rats on the adjusting step (A-B) and vibrissae tests (C-D) the day before, on, and after acute raclopride administration. The arrows represent the day of raclopride administration. The bars show the mean and the error bars represent the standard error of the mean. *p<0.001**

significantly as a result of acute raclopride administration (Day x Group: $F_{2,40}=3.17$, n.s.; Side x Day x Group: $F_{2,40}=0.52$, n.s).

Movement time. The lesion group continued taking longer time to respond to contralateral cues than intact controls (Figure 7.6D-E; Group: $F_{1,20}=25.95$, $p<0.001$; Side x Group: $F_{1,20}=19.83$, $p<0.001$; Simple Effect of Side: $F_{1,20}=21.13$, $p<0.001$). Neither group's movement time increased as a result of acute raclopride administration (Day x Group: $F_{2,40}=1.86$, n.s.; Side x Day: $F_{2,40}=0.80$, n.s.; Side x Day x Group: $F_{2,40}=0.79$, n.s.)

Accuracy. At the time of the raclopride testing cycle, the difference in accuracy originally observed between the intact and lesion groups' had disappeared as a result of the increased accuracy in the lesion group's contralateral responding (Figure 7.6F-G; Group:

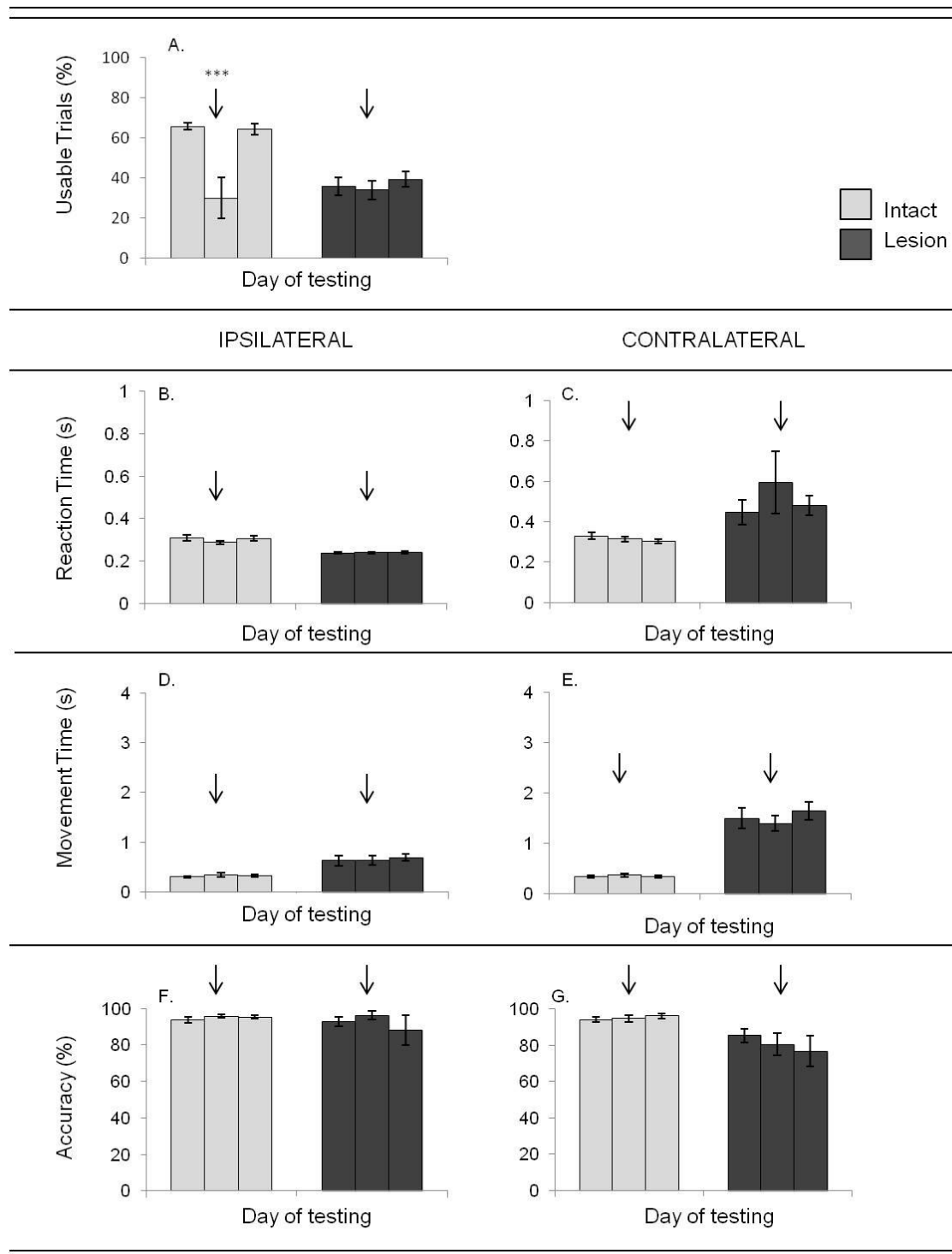


Figure 7.6. The effect of 0.2 mg/kg acute raclopride on intact (n=10) and lesion (n=12) rats' usable trials (A) and ipsilateral and contralateral reaction time (B-C), movement time (D-E), and accuracy (F-G) of intact (n=10) and lesion (n=12) rats following administration of acute bromocriptine. The arrows represent time of raclopride administration. The bars show the mean and the error bars represent the standard error of the mean. *p<0.001**

$F_{1,20}=0.02$, n.s.; Side: $F_{1,20}=0.08$, n.s.; Side x Group $F_{1,20}=4.36$, n.s.). Neither group's accuracy levels were affected by acute raclopride administration (Day: $F_{2,40}=0.046$, n.s.; Day x Group: $F_{2,40}=1.57$, n.s.; Side x Day: $F_{2,40}=0.75$, n.s.; Side x Day x Group: $F_{2,40}=0.31$, n.s.).

7.2.4. Discussion

The primary aim the experiment was to replicate the previously reported gradual decline in contralateral accuracy following unilateral 6-OHDA lesions (Dowd & Dunnett 2004, 2005a,b) and to investigate the neural substrates underpinning the LCRT task deficit by testing the effect of acute bromocriptine on performance. The previous findings of a gradual decline in contralateral accuracy (Dowd & Dunnett, 2004, 2005a,b) was successfully replicated both three and eight weeks post-lesion. The nature of this deficit is discussed in greater detail in the chapter discussion.

In contrast to previous data on the effects of bromocriptine on motor behaviour (Lundblad *et al*, 2002; Lindgren *et al*, 2007), acute administration of 1.25 mg/kg bromocriptine did not improve lesion rats' reaction or movement times on the day of drug administration. This difference may be due to the different paradigms used; whereas the current experiment measured reaction and movement time in the LCRT task on a trial per trial basis, earlier authors demonstrated an effect of bromocriptine on rotational bias over a 180 min (Lindgren *et al*, 2007) or 300 min (Lundblad *et al*, 2002) period. There were thus differences both in regard to the motor behaviour being measured and the time span over which it was measured.

Acute bromocriptine significantly improved the lesion group's contralateral accuracy, which suggests that accurate responding on the LCRT task is primarily D2 driven. Unexpectedly, the drug's beneficial effect on accuracy outlasted its known half-life. Whilst originally aiming to counterbalance drug administration once a therapeutic dose of bromocriptine had been established, thereby allowing a direct comparison between lesion rats tested on and off bromocriptine, the drug's unforeseen long-lasting effect made this impossible. Instead, the effect of bromocriptine was analysed by comparing performance before and after bromocriptine administration. This analysis revealed a significant and long-lasting improvement in the accuracy with which lesion rats responded to contralateral cues post bromocriptine administration. Interestingly, the long-term effects are not likely to have been driven by a continuous stimulation of dopamine receptors as bromocriptine has a half-life of approximately 3h (Lieberman & Goldstein, 1985).

To fully exclude the possibility that the long-term effect was not mediated by stimulation of D2/3 receptors, rats were administered raclopride prior to operant testing. Raclopride is a dopamine antagonist with a high affinity for D2, but also D3, receptors (Table 7.2; Keabian *et al*, 1997). The antagonist successfully reduced motor function, as evidenced by data from the adjusting step and vibrissae tests. However, it did not affect accuracy on the LCRT task. Two conclusions may be drawn from the findings. First, the long-term effect of bromocriptine was unlikely caused by a long-term stimulation of D2 relative to D1 type receptors. Second, because raclopride significantly suppressed motor function without affecting LCRT task accuracy, the data suggest that there is a dissociation between the neurological mechanisms driving motor function and accurate LCRT task responding.

A possible explanation for the long-lasting effect of bromocriptine on accuracy is that acute bromocriptine administration induced downstream changes in the striatal dopaminergic system. However, due to the unexpected effects of bromocriptine, the experimental design did not allow for a lesion bromocriptine naive control group which would have been needed to test this hypothesis. Instead, the hypothesis was tested in a subsequent experiment which included a lesion bromocriptine paired (administered bromocriptine 2 h prior to LCRT task testing), a lesion bromocriptine unpaired (administered bromocriptine 2 h after LCRT task testing), and a lesion bromocriptine naive group. If the effect of bromocriptine described above was due to downstream changes, both the paired and the unpaired group should show improved accuracy after bromocriptine administration, whereas no change should be observed in the contralateral accuracy of the lesion bromocriptine naive group.

A possible second explanation for the phenomenon is that bromocriptine conditioned accurate LCRT task responding. It has previously been shown that rats which have repeatedly been administered bromocriptine in a paired environment exhibit increased activity levels when placed in the paired environment in the absence of bromocriptine (Hoffman & Wise, 1992). This suggests that bromocriptine is able to condition behaviour associated with contextual cues. It could thus be hypothesised that the conditioning effects of bromocriptine extend to also affecting accurate responding on the LCRT task. More specifically, acute administration of bromocriptine may have enhanced accurate contralateral responding on the day of administration by alleviating the effort associated with performing contralateral responses while simultaneously conditioning the drug-induced accurate responding, thereby ultimately causing a long-lasting improvement in the observed contralateral accuracy. This hypothesis may be tested by comparing the contralateral accuracy of lesion rats that are administered bromocriptine prior to (i.e. paired) or after (i.e. unpaired) LCRT task testing.

Due to the unexpected long-lasting effect of bromocriptine the current experimental design did not allow such a test to be conducted. Instead, this hypothesis was tested in the experiment described in the following section.

7.3. Experiment 6 (part II): The effect of acute bromocriptine and chronic L-DOPA on LCRT task performance

7.3.1. Introduction

The primary aim of the current experiment was to replicate the findings of a long-lasting effect of acute bromocriptine on accurate LCRT task responding reported in Experiment 9 whilst including appropriate control groups. To this end, the experiment included both lesion bromocriptine naive rats, lesion rats administered acute bromocriptine 2 h prior to LCRT task testing (paired group), and lesion rats administered acute bromocriptine 2 h post LCRT task testing (unpaired group).

In addition, the experiment also tested the effect of chronic L-DOPA administration on LCRT task performance. Chronic L-DOPA administration causes LID onset in the majority of MFB lesion rats (Winkler *et al*, 2002). LID is associated with increased binding to striatal D1 receptors (Konradi *et al*, 2004; Aubert *et al*, 2005), upregulation of striatal mGlu5 receptors (Sanchez-Pernaute *et al*, 2008; Ouattara *et al*, 2011), and abnormal synaptic plasticity in the cortico-striatal pathway (Picconi *et al*, 2003). As discussed in further detail in the chapter discussion, reduced contralateral accuracy in the LCRT task has previously been hypothesised to be caused by either a decrease in general motivation or extinction of contralateral responding following loss of the dopaminergic reward signal (see Lelos *et al*, 2012). It was hypothesised that if the task was mediated extinction, the regained ability for LTP formation observed in tissue from lesion rats chronically treated with L-DOPA, but not L-DOPA naive rats (Picconi *et al*, 2003) would aid extinction learning (Farinelli *et al*, 2006) and as such induce a further decrease in contralateral accuracy.

7.3.1.2. Aims

The primary aim of the experiment was to replicate the previous findings of a bromocriptine induced improvement in lesion rats' contralateral responding on the LCRT task (Experiment 9) whilst including appropriate lesion control groups. A second aim was to test the effect of chronic L-DOPA treatment on lesion rats' LCRT task performance. Depending on the psychological nature of the task, it was hypothesised that chronic L-DOPA would either not affect behaviour, or further reduce contralateral accuracy.

7.3.2. Methods

7.3.2.1. Experimental design

Rats were trained on the LCRT task before undergoing unilateral 6-OHDA lesions. Following recovery and lesion assessment using the spontaneous rotation and cylinder tests, a subgroup of the lesion rats was chronically treated with L-DOPA until showing stable AIMs. Lesion rats were then tested in staircase boxes and, thereafter, on the LCRT task. Following a second post-lesion LCRT testing cycle, a subgroup from each experimental group was administered acute bromocriptine prior to operant testing (paired group). Remaining rats were given the same bromocriptine dose after LCRT task testing (unpaired group) or kept bromocriptine naive. Following one day of paired or unpaired LCRT task testing, all rats were tested for three more days in the absence of any further drug administrations. The procedure was replicated in two 5 day testing cycles (Figures 7.7 and 7.8).

7.3.2.2. Procedure

The previous experiment used male rats to ensure consistency with the LCRT task publications that I aimed to replicate (Dowd & Dunnett 2004, 2005a,b). Having replicated earlier findings, the subsequent chapters used female rats. This decision was made both on the basis of the practical advantages of using female rats and for scientific reasons. First, female rats are smaller and less aggressive than males, and more rats can therefore be housed in one cage if using females, which decreases the overall holding space required. Second, the rat AIMs scoring system used in this project (Winkler *et al*, 2002) and findings of abnormal plasticity in the cortico-striatal pathway of dyskinetic rats (Picconi *et al*, 2003) were all based on experiments using female rats. Finally, the lesion coordinates used in this thesis project had been shown to produce more stable lesions in smaller rats (Torres *et al*, 2011).

Forty-seven female Lister Hooded rats were pre-trained on the LCRT task for 1 month (section 2.7.2.2) before a subgroup of 37 rats was given unilateral 6-OHDA lesions to the MFB (section 2.2.2). Following a two week recovery period, lesion extent was assessed using the cylinder test and spontaneous rotations (sections 2.2.4 and 2.4.6.3). Twelve of the lesion rats were then administered daily injections of 10 mg/kg L-DOPA (section 2.3.3) for two weeks before being tested on the staircase task for three weeks (section 2.4.4) and,

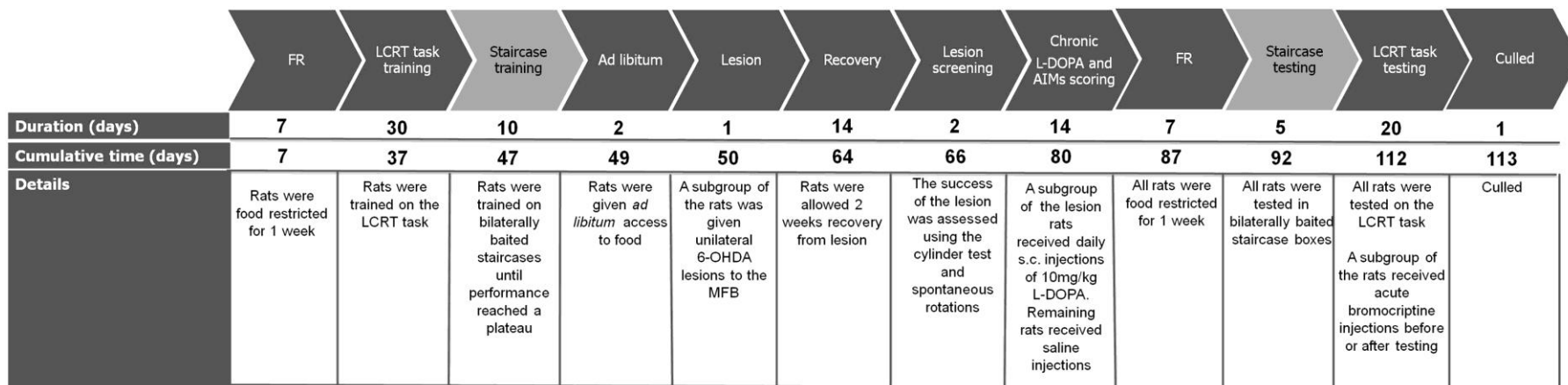


Figure 7.7. A graphical representation of the experimental design in experiment 6. The figure specifies both the duration of the individual testing phases, and the overall number of days in the experiment. Light grey boxes represent testing phases that are described in further detail in section 6.2. BRC=bromocriptine; FR=Food restriction; LCRT task=Lateralised choice reaction time task; MFB=Medial forebrain bundle

finally, on the LCRT task (section 2.7.2.2) for two five-day cycles. The data from the staircase testing are described in section 6.2.

Following an initial five-days post-lesion testing cycle in the absence of any drugs, two further five-day test cycles were conducted in which acute bromocriptine was administered on the second day of each test cycles. In these testing cycles, the L-DOPA naïve lesion rats were split into a drug naïve control group (n=10), a paired group which received bromocriptine 2h before LCRT task testing (n=7), and an unpaired group which received bromocriptine 2h after LCRT task testing (n=8). Hence, only the paired group was tested on the LCRT task while under the influence of bromocriptine. Including lesion BRC naïve, paired, and unpaired rats in the experimental design allowed distinction to be made

between the potential behavioural effects following bromocriptine administration in a specific environment (i.e. conditioning) and potential effects arising from mere bromocriptine exposure (i.e. mediated by downstream changes). Due to limited numbers of rats, the intact rats were only split into a paired (n=5) and drug naïve (n=5) group, as were the L-DOPA treated lesion rats (paired n=6, drug naïve n=6). Figure 7.8 shows the number of rats in each experimental group that were administered acute bromocriptine before or after LCRT task testing. At the end of operant testing, the rats were culled, their striata dissected out, and their hindbrains fixed in 4% PFA. Striatal tissue was gathered to enable later molecular comparison between lesion rats that were bromocriptine naïve, bromocriptine paired, or bromocriptine unpaired. However, as it was not possible to optimise a Western blotting protocol within the time frame of the thesis project such data are not presented.

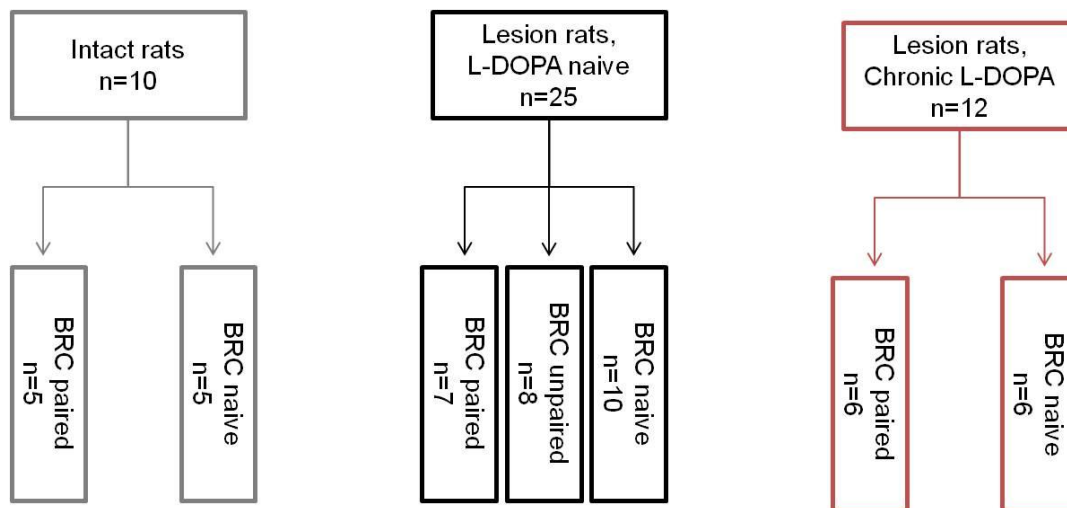


Figure 7.8. The number of rats in the intact (n=10), lesion L-DOPA naïve (n=25), and lesion L-DOPA treated (n=12) groups that remained bromocriptine naïve, or that were administered acute bromocriptine 2h before (paired) or 2h after (unpaired) LCRT task testing. BRC=bromocriptine

7.3.2.3. Statistical analysis

The effect of the lesion on the number of TH+ cell bodies in the SNc and VTA was analysed using two separate types of analyses. First, the presence of a lesion in the SNc or VTA was confirmed by comparing the raw cell counts in the ipsilateral and contralateral hemisphere using a t-test. For this analysis, the SNc and VTA were analysed separately. Once the presence of a lesion had been confirmed, an ANOVA was used to test whether there was a difference in (i) the relative extent of the experimental groups' dopamine denervation, and (ii) the loss of dopaminergic cell bodies observed in the SNc versus the VTA. The ANOVA used

the percentage loss of TH+ cell bodies observed in the lesion SNc and VTA as input data and included Region as a within-subject variable, and Group as a between-subject variable.

AIMs were not normally distributed and analysed using a Mann-Whitney test. All LCRT task data were analysed using a repeated measures ANOVA with Group as between-subject factor, and Side, Cycle, and Day as within-subject factors. Significant between-subject effects were analysed using a Scheffe post-hoc test and significant interactions analysed using a test of simple effects.

7.3.3. Results

7.3.3.1. Effect of lesion & drug treatments

The lesion induced a profound loss of TH+ cell bodies that was greater in the SNc (Figure 7.9A-B; $t_{36}=24.07$, $p<0.001$) than the VTA ($t_{36}=6.84$, $p<0.001$). A further analysis of the data demonstrated that there was a more pronounced loss of dopaminergic cell bodies in the SNc than in the VTA (Region: $F_{1,33}=34.45$, $p<0.001$), and that the dopamine denervation was similar between the experimental groups (Group: $F_{3,33}=0.49$, n.s.; Region x Group: $F_{3,33}=0.30$, n.s.).

The unilateral dopamine loss was reflected in a pronounced ipsilateral bias in rats' spontaneous rotations (Figure 7.9C) and in their performance on the cylinder task (Figure 7.9D). There was no difference between the ipsilateral bias exhibited by the lesion groups on either of these motor tests (Spontaneous rotations: $F_{3,33}=0.45$, n.s.; Cylinder test: $H=1.19$, $df=3$, n.s.), suggesting that the lesion had a similar effect on motor function on all lesion groups.

Following chronic L-DOPA treatment, the lesion L-DOPA group exhibited high and stable AIMs scores in response to acute L-DOPA administration (Figure 7.9E). There was no difference in the magnitude of AIMs observed in lesion, L-DOPA treated rats that would later be allocated to the paired and unpaired bromocriptine groups ($U=16.00$, n.s.).

7.3.3.2. Post-lesion LCRT task performance

Usable trials. While the lesion rats made fewer usable trials than intact controls (Group: $F_{6,40}=12.19$, $p<0.05$), there was no difference between the lesion rats that were later assigned to the bromocriptine naive, bromocriptine paired, or bromocriptine unpaired groups. As neither their usable trials nor their treatment at this point of testing differed, their data were pooled and the analysis repeated only including an intact, a lesion L-DOPA naive, and a lesion L-DOPA treated group in the analysis.

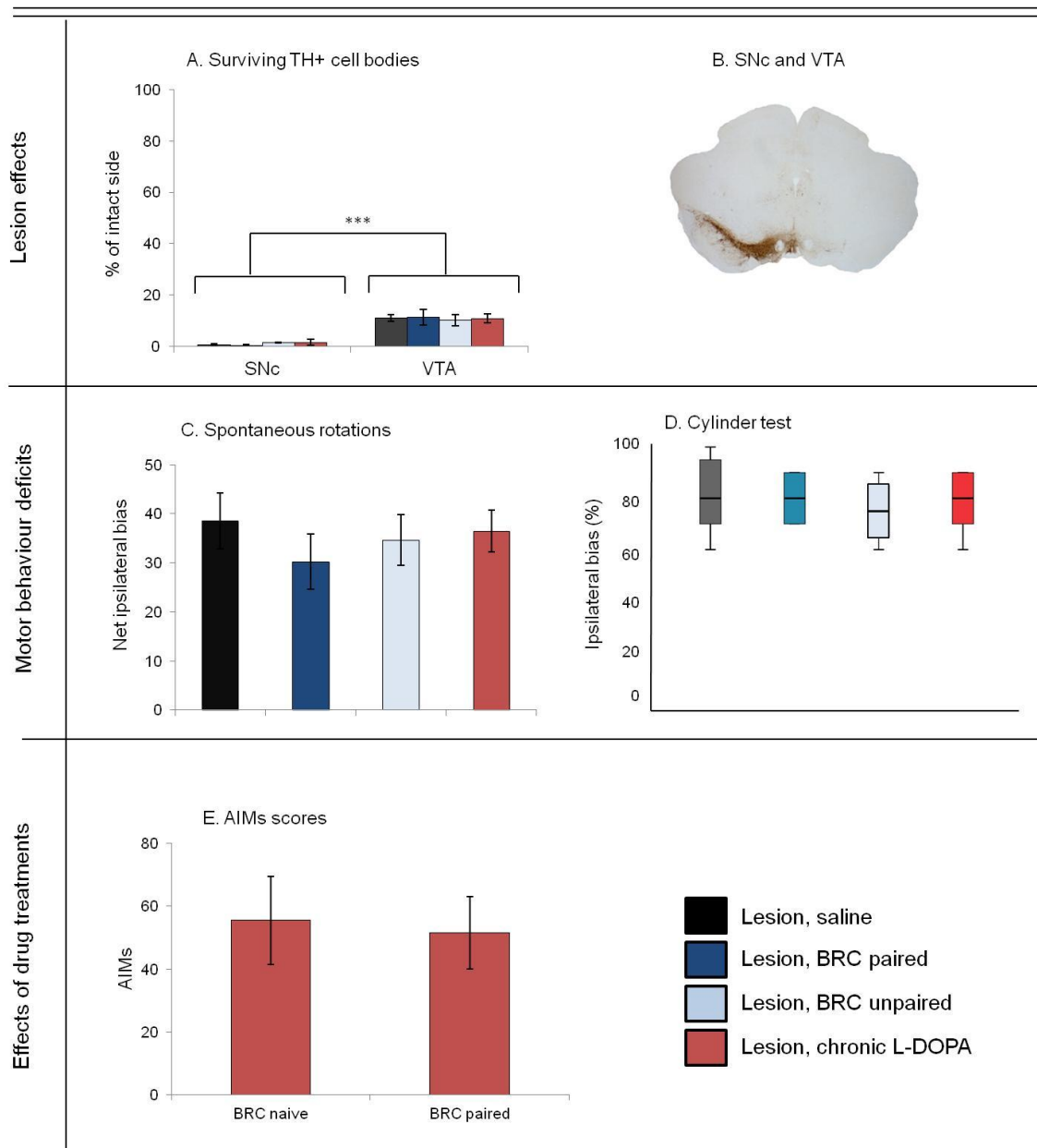


Figure 7.9. Surviving dopaminergic cell bodies in the SNc and VTA, expressed as a percentage of the intact side (A), together with the a photo showing the loss of TH+ cell bodies in the SNc and VTA in one lesion rat included in the experiment (B), the ipsilateral bias exhibited by lesion rats in the spontaneous rotations (C) an cylinder (D) tests during lesion screening, and the cumulative AIMs s scores for the lesion L-DOPA treated rats that remained BRC naive throughout testing or that were later administered acute bromocriptine prior to LCRT testing (E). The bars charts (A, C) show the mean and standard error of the mean. The box plots (D, E) shows the median and the interquartile ranges. BRC=Bromocriptine; SNc=Substantia nigra pars compacta; VTA=Ventra tegmental area; TH=Tyrosine hydroxylase. * $p < 0.001$**

The second analysis demonstrated that Lesion rats conducted overall fewer usable trials than intact controls, but there was no significant difference between the number of usable trials made by the lesion L-DOPA naive and the lesion L-DOPA treated rats (Figure 7.10A; Group: $F_{2,44}=28.77$, $p<0.01$). The number of usable trials made by intact and lesion L-DOPA treated rats fluctuated in the first five-day testing cycle but remained stable in the second post-lesion testing cycle (Cycle x Day x Group: $F_{8,176}=5.06$, $p<0.001$: Simple Effect of Day: min. $F_{4,41}= 3.33$, $p<0.05$).

Reaction Time. There was no difference in the reaction time of the lesion groups that would later be bromocriptine naive, bromocriptine paired, or bromocriptine unpaired (Group: $F_{6,40}=0.31$, n.s.). As neither their reaction time nor their treatment at this point of testing differed their data were pooled and the analysis repeated only including an intact, a lesion L-DOPA naive, and a lesion L-DOPA treated group in the analysis.

Both the lesion groups had longer reaction times than intact controls when cues were presented on their contralateral side. Lesion rats' contralateral reaction time increased over the course of each five-day testing cycle (Figure 7.10B-C; Group: $F_{2,44}=0.87$, n.s; Side x Day x Group: $F_{8,176}=3.32$, $p<0.01$; Simple Effect of Day: $F_{4,41}=\text{min.}6.56$, $p<0.001$). Reactions times fluctuated more in the second than first testing cycle (Cycle: $F_{1,44}=9.68$, $p<0.01$; Cycle x Group: $F_{2,44}=0.90$, n.s.)

Movement Time. There was no difference in the movement time of the lesion groups that would later be bromocriptine naive, bromocriptine paired, or bromocriptine unpaired (Group: $F_{6,40}=1.77$, n.s.). As neither their movement time nor their treatment at this point of testing differed their data were pooled and the analysis repeated only including an intact, a lesion L-DOPA naive, and a lesion L-DOPA treated groups in the analysis.

The second analysis demonstrated that both lesion groups showed longer overall movement time than intact controls (Figure 7.9D-E; Group: $F_{2,44}=15.32$, $p<0.05$). The movement times increased gradually over the five-day testing block (Cycle x Day x Side: $F_{4,176}=3.70$, $p<0.05$; Simple Effect: min. $F_{4,41}=2.84$, $p<0.05$). Movement times were somewhat longer in the second than first than the second post-lesion testing cycle (Cycle: $F_{1,44}=5.27$, $p<0.05$).

Accuracy. There was no difference in the accuracy of the lesion groups that would later be bromocriptine naive, bromocriptine paired, or bromocriptine unpaired (Group: $F_{6,40}=15.40$, $p<0.001$). As neither their accuracy nor their treatment at this point of testing differed their data were pooled and the analysis repeated only including an intact, a lesion L-DOPA naive, and a lesion L-DOPA treated groups in the analysis.

Lesion rats were significantly less accurate in their responding than intact rats, but there was no *overall* difference between lesion rats that were L-DOPA naive or that had received chronic L-DOPA treatment (Figure 7.10F-G; Group: $F_{2,44}=39.48$, $p<0.001$). The lesion groups' deficit manifested itself when rats were required to make contralateral responses, and gradually worsened over each five-day testing cycle (Side x Day x Group: $F_{8,176}=2.86$, $p<0.01$; Simple Effect of Day: min. $F_{4,41}=6.06$, $p<0.01$). The gradual nature of the deficit was most readily observed in the first five-day testing cycle. In the second cycle, lesion rats' contralateral responding did not align with that of intact controls on the first day of testing thus making the gradual nature of the deficit less pronounced (Cycle x Side x Day: $F_{4,176}=5.46$, $p<0.05$; Simple Effect of Cycle: min. $F_{1,44}=5.05$, $p<0.05$).

Because the deficit primarily manifested itself on the contralateral side a further analysis, restricted to the contralateral side, was conducted. As in the previous analysis, the lesion groups were less accurate than intact controls and their performance gradually decreased over the five days of testing (Day x Group: $F_{8,176}=6.32$, $p<0.001$; Simple Effect of Day: min. $F_{4,41}=6.06$, $p<0.01$) both when tested three and eight weeks post-lesion (Day x Group x Cycle: min. $F_{1,41}=9.82$, $p<0.001$). In addition, and in contrast to the analysis including both sides, there was a significant difference between lesion L-DOPA naive and L-DOPA treated rats with chronic L-DOPA treatment further impairing the accuracy with which lesion rats responded to contralateral cues (Group: $F_{2,44}=60.00$, $p<0.001$).

7.3.3.3. Effect of acute BRC on LCRT task performance

At the end of the second post-lesion testing cycle a subset of each experimental group ('paired' groups) was administered 1.25 mg/kg bromocriptine 2 h prior to LCRT testing. The following section describes the effect of paired bromocriptine administration on LCRT task performance. While the statistical analysis included all groups, the data from the L-DOPA naive and L-DOPA treated lesion rats are shown in separate graphs to increase the ease with which the graphs can be read. Similarly, while the statistical analysis did not pool data from intact rats that were bromocriptine naïve or paired, data from these rats are shown as one group in the graphs to increase the ease with which the graphs can be read.

Usable trials. Lesion rats conducted fewer usable trials than intact controls in both five-day testing cycles (Figure 7.11; Cycle: $F_{1,6}=0.43$, n.s.; Cycle x Group: $F_{6,40}=1.07$, n.s.; Group: $F_{6,40}=10.08$, $p<0.001$). In neither cycle did acute bromocriptine significantly increase the number of usable trials conducted at the day of administration (Day: $F_{4,160}=1.19$, n.s.; Day x Group: $F_{24,160}=1.13$, n.s.).

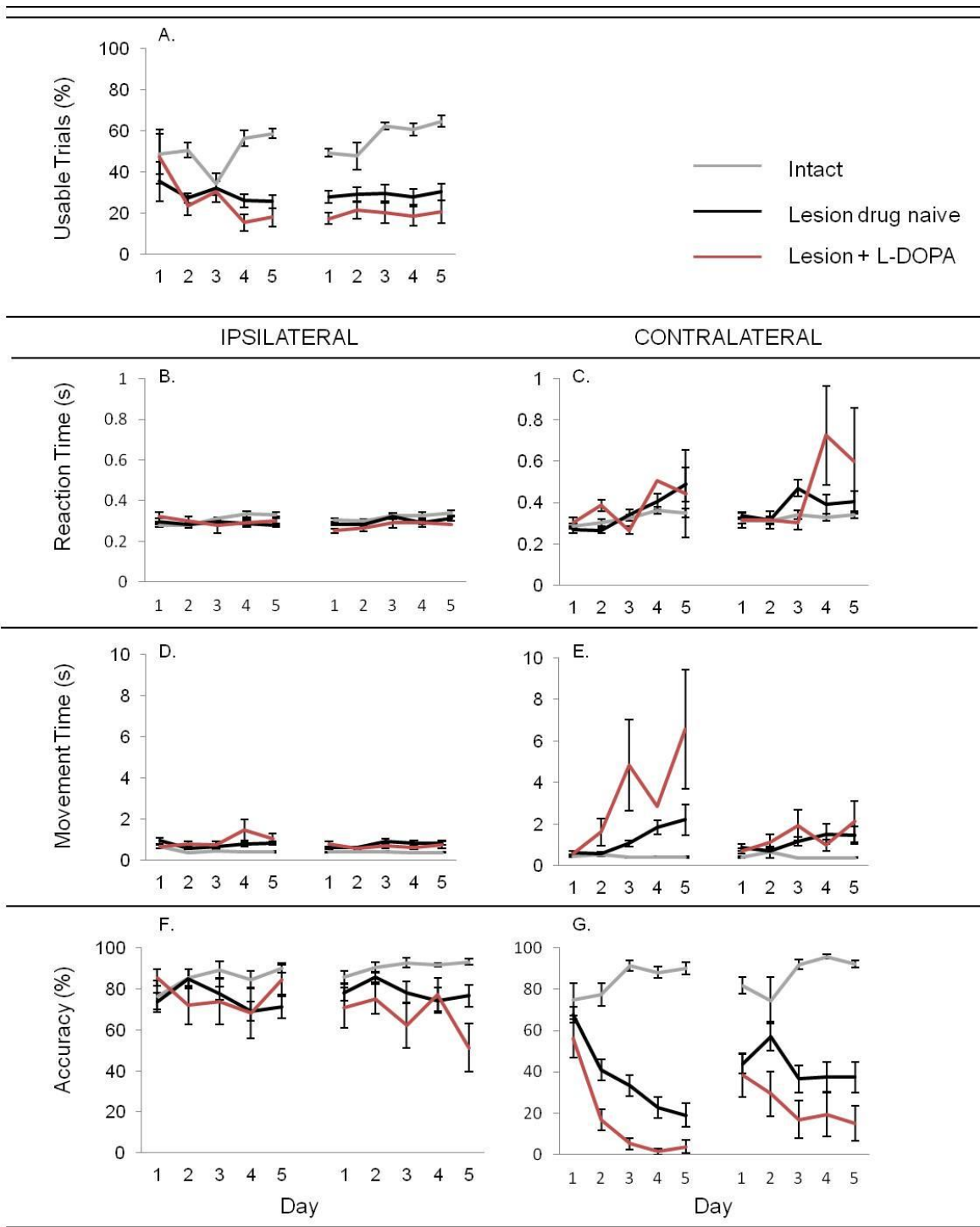


Figure 7.10. The post-lesion usable trials (A) together with ipsilateral and contralateral correct reaction time (B-C), movement time (D-E), and accuracy (F-G) of intact (n=10), lesion drug naïve rats (n=25), and lesion rats that had been chronically treated with L-DOPA (n=12). The lines show the mean and the error bars represent the standard error of the mean.

Reaction Time. There was no overall difference in intact and lesion groups' reaction times (Figure 7.12; Group: $F_{6,40}=1.56$, n.s.). While there was a trend towards longer reaction times on the contralateral than ipsilateral side this did not meet statistical significance (Side: $F_{1,40}=3.73$, $p=0.06$; Side x Group: $F_{6,40}=1.85$, n.s.). Reaction times were of similar length in the first and second five day testing cycle (Cycle: $F_{1,40}=1.45$, n.s.; Cycle x Group: $F_{6,40}=0.61$, n.s.). Together, these data indicate that acute bromocriptine administration did not significantly affect intact or lesion rats' reaction times.

USABLE TRIALS

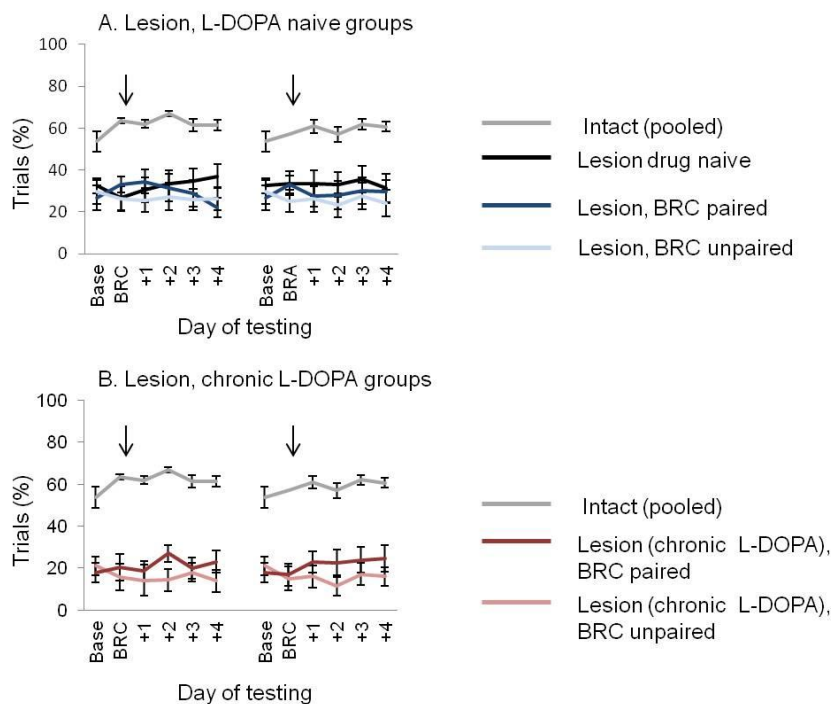


Figure 7.11. The percentage of usable trials conducted by intact rats, lesion L-DOPA naive (top row), and lesion rats that had been chronically treated with L-DOPA (bottom row). A subset of rats were administered acute bromocriptine 2 h prior to testing (paired group) or 2 h after testing (unpaired group) on the second day of each five-day testing cycle. The arrows indicate the day of acute bromocriptine administration. The lines show the mean and the error bars show the standard error of the mean. Base=baseline, BRC=bromocriptine

Movement Time. The lesion groups took longer time than intact controls to respond to cues presented on their contralateral side (Figure 7.13; Side x Group: $F_{6,40}=4.61$, $p<0.01$; Simple Effect of Side: $\min.F_{1,40}=8.94$, $p<0.01$). While the lesion, bromocriptine paired group' movement time fluctuated over the course of testing, these fluctuations were not specific to

the days of bromocriptine administration (Group x Day: $F_{24,160}=1.65$, $p<0.05$; Simple Effect of Day: $F_{4,37}=5.41$, $p<0.01$), suggesting that bromocriptine did not significantly affect movement times. The movement times were similar in the first and second five-day testing cycles (Cycle: $F_{1,40}=0.53$, n.s.; Cycle x Group: $F_{6,40}=0.26$, n.s.).

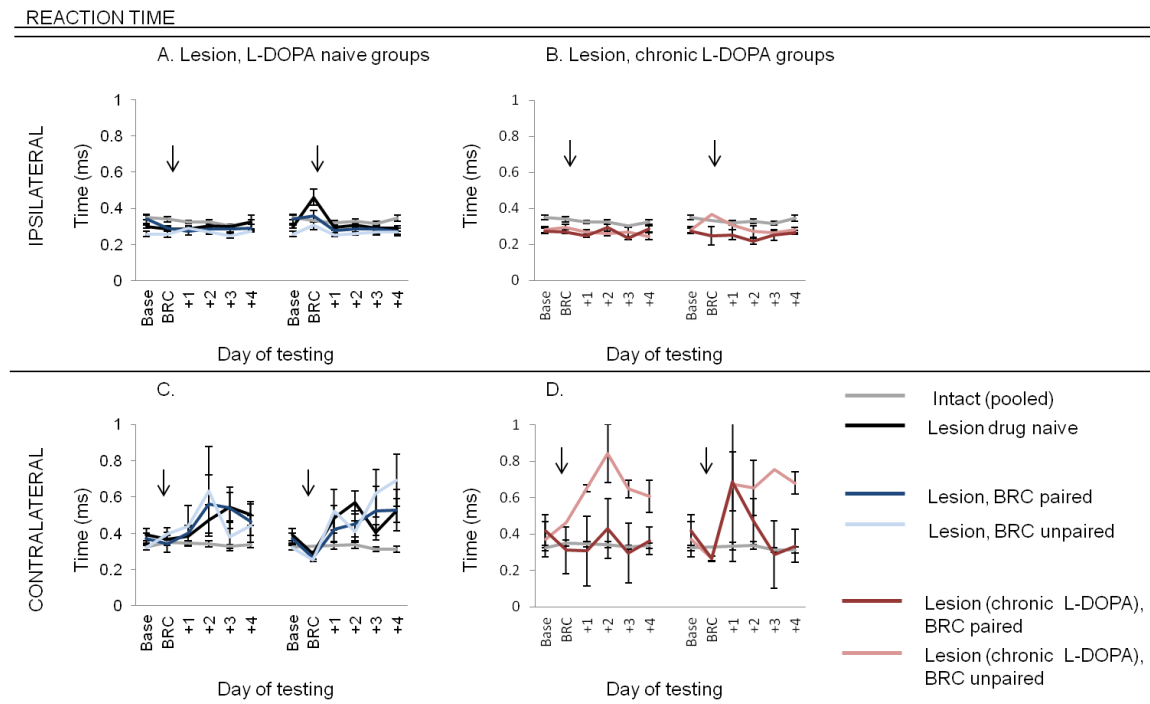


Figure 7.12. The ipsilateral (top row) and contralateral (bottom row) reaction times exhibited by intact rats, lesion L-DOPA naive rats (left column), and lesion rats that had been chronically treated with L-DOPA (right column). A subset of rats were administered acute bromocriptine 2 h prior to testing (paired group) or 2 h after testing (unpaired group) on the second day of each five-day testing cycle. The arrows indicate the day of acute bromocriptine administration. The lines show the mean and the error bars show the standard error of the mean. Base=baseline, BRC=bromocriptine

Accuracy. The lesion groups were, overall, less accurate in their responding than intact controls (Figure 7.14; Group: $F_{6,40}=6.30$, $p<0.001$). This lesion deficit manifested itself when rats responded to contralateral cues (Side x Group: $F_{6,40}=6.77$, $p<0.001$; Simple Effect: $F_{6,40}=8.71$, $p<0.0001$). The accuracy with which rats responded to cues presented on their contralateral side changed over the course of testing exclusively in the two lesion groups that received acute bromocriptine two hours prior to LCRT task (Side x Day x Group: $F_{24,160}=2.18$, $p<0.01$; Effect of Day on paired lesion groups: $\min.F_{4,37}=4.99$, $p<0.01$). However, while acute bromocriptine increased accuracy on the day of administration, there

was no long-lasting effect of the drug on accuracy as had been observed in Experiment 9. Overall accuracy was significantly higher in the second (average: 64.12%) than first (average: 59.33%) five-day testing block (Cycle: $F_{1,40}=4.83$, $p<0.05$; Cycle x Group: $F_{6,40}=0.84$, n.s.).

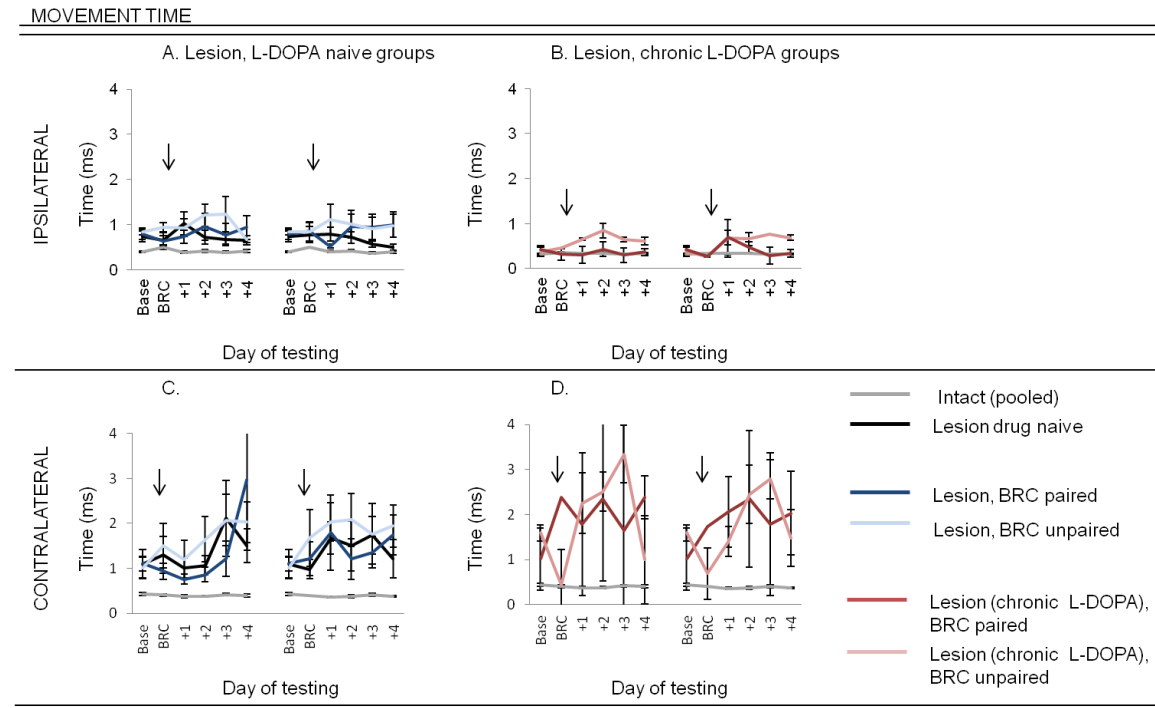


Figure 7.13. The ipsilateral (top row) and contralateral (bottom row) movement time exhibited by intact rats, lesion L-DOPA naive rats (left column), and lesion rats that had been chronically treated with L-DOPA (right column). A subset of rats were administered acute bromocriptine 2h prior to testing (paired group) or 2h after testing (unpaired group) on the second day of each 5 day testing cycle. The arrows indicate the day of acute bromocriptine administration. The lines show the mean and the error bars show the standard error of the mean. Base=baseline, BRC=bromocriptine

7.3.4. Discussion

The primary aim of the experiment was to determine whether acute bromocriptine administration had long-lasting effects on contralateral accuracy, as indicated in Experiment 9 (section 7.2). Whilst the drug produced a transient increase in contralateral accuracy, the current data failed to replicate the long-lasting effect on accuracy that was observed in the previous section. The difference may be by due to a type I error in Experiment 9, or methodological differences in the experiments.

Whilst Experiment 9 used male rats in an attempt to follow the original LCRT task protocol (Dowd & Dunnett, 2004) as closely as possible, the current experiment used female

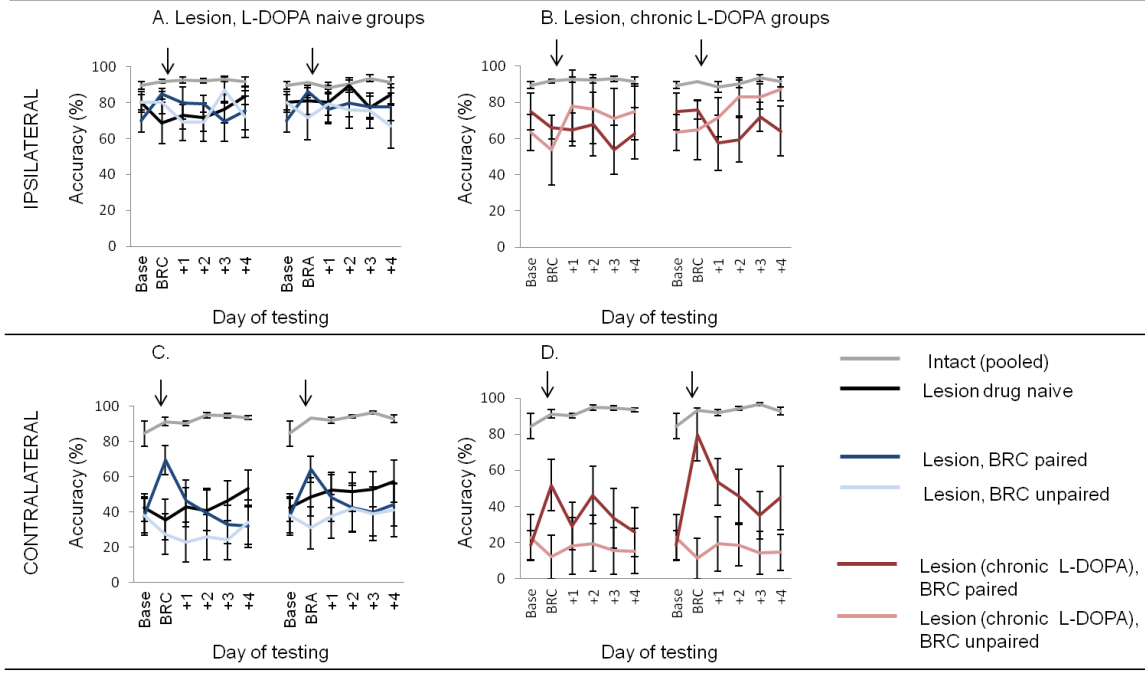


Figure 7.14. The ipsilateral (top row) and contralateral (bottom row) accuracy exhibited by intact rats, lesion L-DOPA naive (left column), and lesion rats that had been chronically treated with L-DOPA (right column). A subset of rats were administered acute bromocriptine 2 h prior to testing (paired group) or 2 h after testing (unpaired group) on the second day of each 5 day testing cycle. The arrows indicate the day of acute bromocriptine administration. The lines show the mean and the error bars show the standard error of the mean. Base=baseline, BRC=bromocriptine

rats for practical reasons and for consistency with other experiments reported in this thesis. There are demonstrated gender differences in the human dopaminergic system in terms of D2 binding and the adverse effects of dopamine agonists (Kaasinen, 2001; Munro et al, 2006; Joutsa et al, 2012). The possibility that gender differences in the psychopharmacological effects of bromocriptine translate to the rat cannot be excluded and may explain the different results obtained in this and in the previous section.

It should also be noted that the rats in this experiment showed greater loss of dopaminergic cell bodies than the rats in Experiment 9. This was likely an indirect effect of the gender difference, as the lesion coordinates used in the experiments have been optimised for female rats that are smaller than the male rats used in Experiment 9 (Torres *et al*, 2011). It was previously hypothesised that the long-lasting effect of acute bromocriptine administration on lesion rats' contralateral responding was due to conditioning. It is possible

that, in order for a conditioning effect to occur, there must be more surviving dopaminergic fibres than what was present in the current experiment.

The data were, however, valuable insofar as providing further insight into the mechanism underlying the contralateral accuracy deficit. In line with what had been expected in Experiment 9, bromocriptine administration induced a transient increase in the contralateral accuracy of both drug naïve and L-DOPA treated lesion rats. Interestingly, the chosen bromocriptine dose was able to improve lesion rats' accuracy whilst no effect was observed on reaction or movement times. Thus, the data supports earlier findings that accurate responding in the LCRT task is D2 reliant and that it is dissociated from motor aspects of the LCRT task.

A main finding was the further decrease in contralateral accuracy observed in the lesion L-DOPA treated group. Whilst long-term L-DOPA treatment is known to induce adverse side effects in the form of AIMs in lesion rats and patients these are of a motor nature and are only present when on medication. In the current experiment, chronic L-DOPA treatment was shown to decrease contralateral accuracy in lesion rats tested in the absence of acute L-DOPA. The data thus suggests that chronic L-DOPA medication might have long-term effects on non-motor function, something which is further discussed in the chapter discussion. The findings of an effect of chronic L-DOPA treatment on LCRT task performance were followed up in two subsequent experiments described in sections 7.4 and 8.2.

7.4. Experiment 8 (part II): The Effect of Chronic L-DOPA and Chronic Bromocriptine Treatment on LCRT Task Performance

7.4.1. Introduction

Previous findings (section 7.3) suggested that chronic L-DOPA treatment further impaired lesion rats' accuracy on the LCRT task. In addition to L-DOPA, D2 agonists are also used to treat motor symptoms of PD and unlike L-DOPA, these do not induce LID if administered as a first-line monotherapy (Perace *et al*, 1998). As further elaborated on in the chapter discussion, it was hypothesized that the previously observed L-DOPA induced deficit was linked to changes in synaptic plasticity which occurs in lesion rats following chronic L-DOPA (Picconi *et al*, 2003). To test the hypothesis, while also allowing a comparison between the effects of common dopaminergic PD treatments on motor and non-motor function, the current experiment tested whether chronic treatment with the D2 type agonist

bromocriptine would induce a similar impairment as chronic L-DOPA. Bromocriptine was chosen as the D2 agonist to be tested both because previous finding (sections 7.2 to 7.3) had shown a beneficial effect of acute bromocriptine on lesion rats' non-motor performance in the LCRT task, and because it does not induce LID in drug naive lesion rats (Pearce *et al*, 1998). Including bromocriptine in the current experiment thus allowed a comparison to be made between (i) acute (as demonstrated in sections 7.2 and 7.3) and chronic effects of bromocriptine on LCRT task performance, and (ii) the chronic effect of LID inducing (L-DOPA) and non-LID inducing (bromocriptine) drugs on LCRT task performance. While testing the acute effects of bromocriptine and L-DOPA was not a primary aim of the experiment, the opportunity was seized to also test the ability of acute L-DOPA and bromocriptine to alleviate lesion induced deficits on the LCRT task at the end of testing.

7.4.1.2. Aims

The aims of the experiment were to (i) test the effect of chronic L-DOPA and bromocriptine treatment on intact and lesion rats' LCRT task performance, and (ii) test the ability of acute L-DOPA and bromocriptine to improve LCRT task performance in lesion rats with a previous history of L-DOPA or bromocriptine treatment.

7.4.2. Methods

7.4.2.1. Experimental design

Rats were trained on the LCRT task before a subgroup was given unilateral 6-OHDA MFB lesions. Following recovery, lesion extent was assessed using the cylinder test and spontaneous rotations. Intact and lesion rats were then divided into three groups and chronically treated with saline, L-DOPA, or bromocriptine respectively. After treatment, rats were tested in staircase boxes (reported in section 6.4) and on the LCRT task in the absence of any drugs. Following LCRT testing off-medication, rats were also tested in the LCRT task following acute administration of the drug with which they had previously been treated (Figure 7.15). Striatal tissue had originally been intended to be used for molecular analysis, it was not therefore not appropriate to administer rats more than one type of injection (i.e. saline, L-DOPA, or bromocriptine). Therefore, the effect of acute bromocriptine was only tested in rats that had previously received chronic bromocriptine, and the effect of acute L-DOPA was only tested in rats that had received chronic L-DOPA.

7.4.2.2. Procedure

Eighty-five female Lister Hooded rats were trained on the LCRT task (section 2.7.2.2) for six weeks before 58 rats were given unilateral 6-OHDA lesions to the MFB (section 2.2.2). Two

weeks post-lesion, the lesion extent was assessed using the cylinder test (section 2.4.4.) and by measuring rats' spontaneous rotations (section 2.4.6.3). Intact and lesion rats were then chronically treated with 1 ml/kg saline (intact n=10, lesion n=15), 10 mg/kg L-DOPA (intact n=10, lesion n=25), or 1.25 mg/kg bromocriptine (intact n=10, lesion n=15). Chronic treatment lasted for three weeks, during which period rats received daily injections of their respective drug. The lesion L-DOPA and bromocriptine treated groups were scored for AIMs throughout the treatment period (section 2.5). The behavioural effect of bromocriptine was also assessed twice weekly by measuring lesion rats' rotational bias in automated rotometers (section 2.4.6) for 90 min post-injection. Saline treated rats were not scored for AIMs.

At the end of the chronic drug treatment, rats were tested in staircase boxes for three weeks (section 6.4), during which period weekly maintenance injections of the groups' respective drugs were provided at the same dose as before. To demonstrate that the dyskinetic effect of L-DOPA was not lost during this period, the effects of acute L-DOPA and bromocriptine were measured again at the end of staircase testing, using the same protocol as during chronic drug treatment.

Rats were thereafter tested on the LCRT task, in the absence of any drugs for 5 days before a second testing cycle measuring the effect of acute drug administration commenced. In the acute testing cycle, intact and lesion rats were given acute administrations of the same drug they had been chronically treated with, either before (paired group) or after (unpaired group) LCRT task testing. Bromocriptine (section 2.3.1) was administered i.p. at a concentration of 1.25 mg/kg and a volume of 2 ml/kg 2 h before/after testing, whereas 1 mg/kg saline and 1 mg/kg L-DOPA (2.3.2) was administered s.c. at a volume of 1 ml/kg either 20 min before or 2h after testing.

The intention was to use striatal tissue from rats treated with saline, L-DOPA, or bromocriptine to determine if there were differences in the molecular effects the drugs produced. To not confound later molecular analysis, it was necessary to only expose rats to one single drug treatment. For this reason, the acute effects of saline, L-DOPA and bromocriptine were only tested in rats that had previously been treated with the same drug. Unfortunately, it was not however possible to optimise the relevant protocol and run such analyses within the time frame of this thesis project and molecular data are therefore not reported in this section.

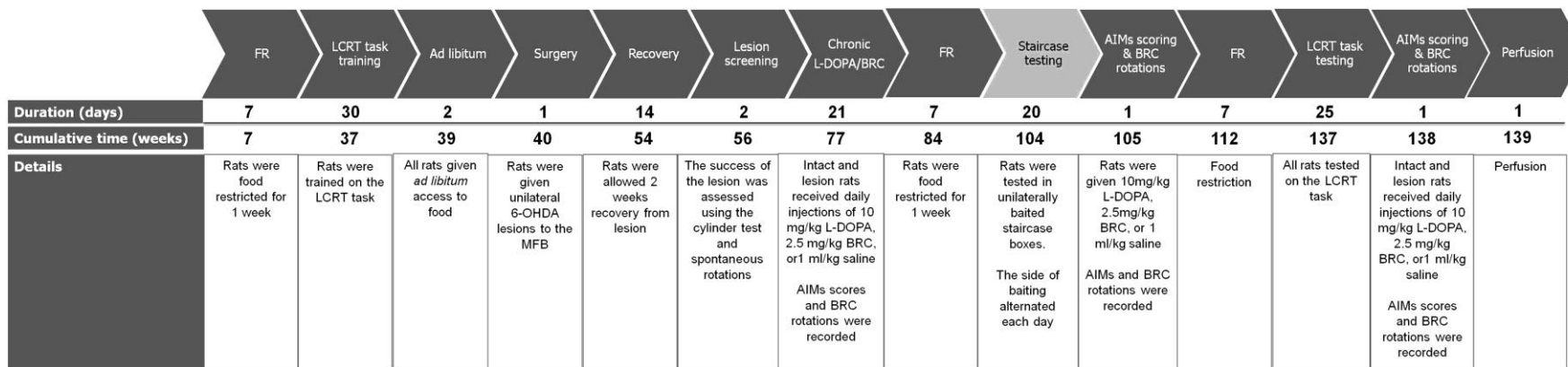


Figure 7.15. A graphical representation of the experimental design. The figure specifies both the duration of the individual testing phases, and the overall number of days in the experiment. Light grey boxes indicate testing that was conducted as part of another experiment and which is further described in section 6.4. BRC=bromocriptine; FR=Food restricted; LCRT task=Lateralised choice reaction time task; MFB=Medial forebrain bundle

One intact rat treated with L-DOPA was excluded from acute administration testing cycle due to health reasons. Hence, the following groups were included in the acute challenge testing cycle: intact paired/unpaired saline (n=5/4), lesion paired/unpaired saline (n=7/8), intact paired/unpaired L-DOPA (n=5/5), lesion paired/unpaired L-DOPA (n=13/12), intact paired/unpaired bromocriptine (n=5/5), lesion paired/unpaired bromocriptine (n=7/8).

At the end of the acute testing cycle, rats were euthanized by an overdose of Euthatal, their hindbrains were post-fixed in 4% PFA, and their striata dissected out (section 2.9) placed on dry ice and thereafter moved to -80°C. Immunohistological staining for TH and counting of cell bodies in the ipsilateral and contralateral SNc and VTA was used to determine lesion extent as described in sections 2.10 and 2.11.

While the striatal tissue was originally kept for later Western blotting tests, it was not possible to optimise the Western blot protocol during the time of the thesis project and data from striatal tissue is therefore not reported.

7.4.2.2. Statistical analysis

The effect of the lesion on the number of TH+ cell bodies in the SNc and VTA was analysed using two separate types of analyses. First, the presence of a lesion in the SNc or VTA was confirmed by comparing the raw cell counts in the ipsilateral and contralateral hemisphere using a t-test. For this analysis, the SNc and VTA were analysed separately. Once the presence of a lesion had been confirmed, an ANOVA was used to test whether there was a difference in (i) the relative extent of the experimental groups' dopamine denervation, and (ii) the loss of dopaminergic cell bodies observed in the SNc versus the VTA. The ANOVA used the percentage loss of TH+ cell bodies observed in the lesion SNc and VTA as input data and included Region as a within-subject variable, and Group as a between-subject variable.

Data from the spontaneous rotations and cylinder tests were analysed using a one-way ANOVA and a Kruskal-Wallis test, respectively. Changes in the net bias of bromocriptine-induced rotations were analysed using a repeated measures ANOVA, where the net rotational bias from the first and last rotometer session in the chronic treatment phase were used as within-subject factors. AIMs scores were analysed using a one-way ANOVA.

LCRT operant data were analysed using a repeated measures ANOVA. Significant between-subject differences were analysed using the Scheffe post-hoc test, and significant interactions analysed using a test of simple effects.

7.4.3. Results

7.4.3.1. Effect of lesion and drug treatments

The lesion induced a significant loss of dopaminergic cell bodies in the ipsilateral SN (Figure 7.16A-B; $t_{54}=30.98$, $p<0.001$) and VTA ($t_{54}=31.02$, $p<0.001$). A further analysis demonstrated that there was a greater percentage loss of dopaminergic cell bodies in the ipsilateral SNc than in the ipsilateral VTA (Region: $F_{1,51}=26.43$, $p<0.001$) and that the dopamine denervation was similar between the groups (Group: $F_{3,51}=0.66$, n.s.; Region x Group: $F_{3,51}=0.39$, n.s.).

Reflective of their lesion, all groups showed an ipsilateral bias on the spontaneous rotations and cylinder tests (Figure 7.16C-D) that did not differ between the three groups (Spontaneous rotations: $F_{2,52}=1.55$, n.s.; Cylinder test: $H(2)=0.74$, n.s.). The lesion group

treated with L-DOPA exhibited high AIMs scores following chronic treatment (Figure 7.16E), something which was not observed in the lesion group treated with bromocriptine (Group: $F_{1,39}=16.97$, $p<0.001$). Chronic bromocriptine did, however, produce an increase in the net bias of rats' drug-induced rotations (Figure 7.16F; $F_{1,15}=12.39$, $p<0.01$).

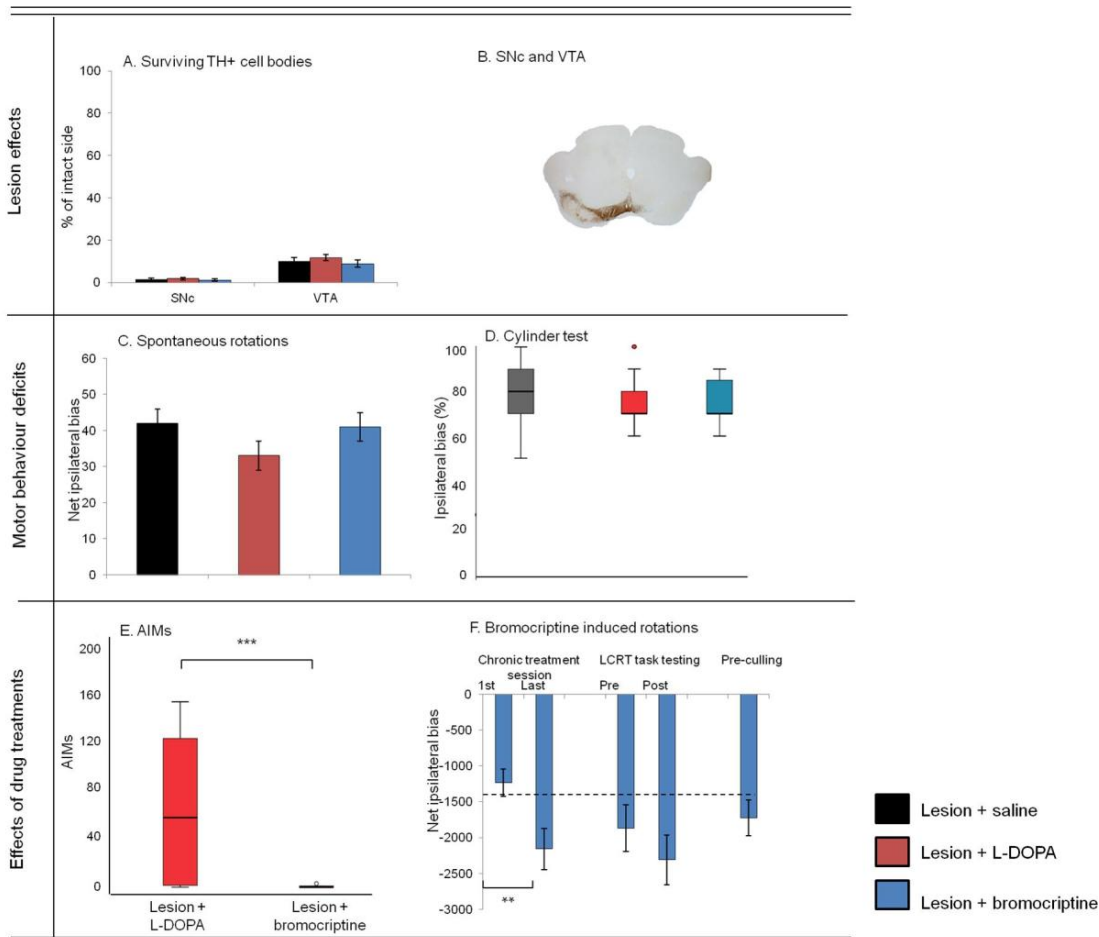


Figure 7.16. The top row shows the percentage of surviving TH+ cell bodies in the SNc and VTA, relative to the intact side, (A) and a photograph of the dopamine depletion in the same neural regions of one lesion rat included in the experiment (B). The middle row shows the net ipsilateral bias of rats' spontaneous rotations (C), and the ipsilateral bias on the cylinder test (D) post lesion but prior to drug treatment. The bottom row shows the cumulative AIMs scores from the final scoring session (E), together with the net ipsilateral bias of bromocriptine induced rotations observed in the lesion = bromocriptine treated group (F). The dotted line in graph F represents the rotational bias exhibited on the first test session. The bars charts (A,C, F) show the mean and the error bars the standard error of the mean. The box plots (D,E) show the median and the interquartile ranges, with circles representing outliers. AIMs=Abnormal involuntary movements; SNc=Substantia nigra pars compact; TH=Tyrosine hydroxylase; VTA=Ventral tegmental area. ** $p<0.01$

7.4.3.2. Post-lesion performance on the LCRT task

There was no significant effect of drug treatment on intact rats' performance, and data from these groups were therefore pooled in subsequent analyses. Four groups were thus included in the analysis; intact controls, lesion + saline; lesion + L-DOPA; and lesion + bromocriptine. For transparency, the LCT task data from the intact groups is shown in Table 7.3 to Table 7.6 and the results from the statistical analysis including all intact subgroups are shown in Appendix I.

Usable Trials. Lesion rats produced fewer usable trials than intact controls but there was no effect of drug treatment (Figure 7.17A; Group: $F_{3,81}=29.42$, $p<0.001$). The number of usable trials made increased over the course of testing (Day: $F_{4,324}=105.05$, $p<0.001$).

Table 7.3. The mean percentage of usable trials performed by the intact groups chronically treated with saline, L-DOPA, or bromocriptine during the first five days of post-lesion testing. The standard error of the mean is shown after each value. BRC=bromocriptine

| Usable trials (%) | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |
|-------------------|------------|------------|------------|------------|------------|
| Intact + saline | 21.0 ± 2.5 | 35.9 ± 4.0 | 46.5 ± 4.6 | 44.2 ± 3.9 | 54.7 ± 3.1 |
| Intact + L-DOPA | 23.2 ± 2.2 | 33.5 ± 4.4 | 46.8 ± 4.7 | 50.7 ± 4.7 | 51.7 ± 4.7 |
| Intact + BRC | 18.7 ± 3.7 | 34.5 ± 3.5 | 42.3 ± 4.8 | 47.4 ± 3.3 | 48.8 ± 2.6 |

Table 7.4. The mean reaction time (s) exhibited by the intact groups chronically treated with saline, L-DOPA, or bromocriptine during the first five days of post-lesion testing. The standard error of the mean is shown after each value. BRC=bromocriptine

| Reaction time (s) | Ipsilateral | | | | | Contralateral | | | | |
|-------------------|-------------|-------------|-------------|-------------|-------------|---------------|-------------|-------------|-------------|-------------|
| | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |
| Intact + saline | 0.28 ± 0.01 | 0.28 ± 0.01 | 0.29 ± 0.01 | 0.30 ± 0.02 | 0.30 ± 0.01 | 0.26 ± 0.01 | 0.28 ± 0.01 | 0.29 ± 0.01 | 0.28 ± 0.01 | 0.31 ± 0.01 |
| Intact + L-DOPA | 0.26 ± 0.02 | 0.31 ± 0.01 | 0.32 ± 0.01 | 0.30 ± 0.03 | 0.34 ± 0.03 | 0.28 ± 0.02 | 0.30 ± 0.02 | 0.32 ± 0.02 | 0.30 ± 0.01 | 0.32 ± 0.02 |
| Intact + BRC | 0.35 ± 0.04 | 0.32 ± 0.05 | 0.32 ± 0.03 | 0.33 ± 0.03 | 0.33 ± 0.03 | 0.30 ± 0.02 | 0.32 ± 0.05 | 0.31 ± 0.03 | 0.31 ± 0.02 | 0.31 ± 0.02 |

Table 7.5. The mean movement time (s) exhibited by the intact groups chronically treated with saline, L-DOPA, or bromocriptine during the first five days of post-lesion testing. The standard error of the mean is shown after each value. BRC=bromocriptine

| Movement time (s) | Ipsilateral | | | | | Contralateral | | | | |
|-------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |
| Intact + saline | 0.81 ± 0.17 | 0.50 ± 0.05 | 0.49 ± 0.07 | 0.45 ± 0.05 | 0.43 ± 0.06 | 0.65 ± 0.07 | 0.55 ± 0.21 | 0.45 ± 0.03 | 0.38 ± 0.02 | 0.38 ± 0.02 |
| Intact + L-DOPA | 0.69 ± 0.08 | 0.55 ± 0.10 | 0.47 ± 0.05 | 0.38 ± 0.02 | 0.46 ± 0.06 | 0.64 ± 0.08 | 0.46 ± 0.02 | 0.49 ± 0.04 | 0.40 ± 0.02 | 0.37 ± 0.02 |
| Intact + BRC | 1.37 ± 0.51 | 0.58 ± 0.05 | 0.59 ± 0.09 | 0.44 ± 0.03 | 0.42 ± 0.03 | 0.88 ± 1.20 | 0.53 ± 0.06 | 0.55 ± 0.08 | 0.44 ± 0.03 | 0.42 ± 0.03 |

Table 7.6. The mean percent accurate trials conducted by the intact groups chronically treated with saline, L-DOPA, or bromocriptine during the first five days of post-lesion testing. The standard error of the mean is shown after each value. BRC=bromocriptine

| Accuracy (%) | Ipsilateral | | | | | Contralateral | | | | |
|-----------------|----------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |
| Intact + saline | 69.1 ± 5.3 | 81.0 ± 2.3 | 82.4 ± 6.4 | 87.1 ± 4.1 | 87.3 ± 3.3 | 74.1 ± 3.7 | 82.1 ± 5.0 | 84.1 ± 3.0 | 86.0 ± 3.9 | 90.0 ± 2.2 |
| Intact + L-DOPA | 59.2 ± 7.5 | 78.2 ± 3.8 | 72.4 ± 5.7 | 82.3 ± 3.1 | 86.4 ± 4.1 | 69.6 ± 4.1 | 80.6 ± 3.5 | 86.9 ± 4.9 | 82.5 ± 2.1 | 86.1 ± 2.0 |
| Intact + BRC | 50.2 ± 10.2 | 69.0 ± 9.0 | 81.1 ± 6.7 | 81.9 ± 6.1 | 80.8 ± 5.6 | 62.6 ± 5.8 | 80.1 ± 4.3 | 80.1 ± 5.2 | 86.7 ± 2.7 | 88.6 ± 3.2 |

Reaction time. While there was no overall difference in the groups' reaction times (Figure 7.17B-C; Group: $F_{3,81}=0.93$, n.s.) the lesion groups exhibited marginally longer reaction times than intact controls when the cues were presented on their contralateral side (Side x Group: $F_{3,81}=7.03$, $p<0.001$; Simple Effect: $F_{3,81}=3.40$, $p<0.05$). The reaction times fluctuated over the course of testing (Day: $F_{4,324}=5.67$, $p<0.001$). However, these fluctuations were not specific to any group (Group x Day: $F_{12,324}=1.13$, n.s.) or side (Side x Day:

$F_{4,324}=1.67$, n.s.; Side x Day x Group: $F_{12,324}=1.17$, n.s.) which suggested that they were due to random fluctuations in the data rather than reflective of a lesion or drug induced deficit.

Movement time. The intact controls had significantly shorter overall movement time than lesion rats. In addition, lesion rats chronically treated with L-DOPA exhibited overall longer movement times than lesion rats treated with saline (Figure 7.17D-E; Group: $F_{3,81}=21.85$, $p<0.0001$). This deficit was most evident when rats were required to respond to visual cues presented on their contralateral side (Side x Group: $F_{3,81}=7.84$, $p<0.001$; Simple Effect of Side on L-DOPA group: $F_{3,81}=35.98$, $p<0.001$). A second analysis, restricted to the contralateral side, was therefore conducted. This analysis demonstrated that the lesion groups treated with L-DOPA and bromocriptine had longer movement times than intact controls (Group: $F_{3,81}=3.51$, $p<0.05$) and that the lesion L-DOPA rats showed a gradual increase in the time required to perform contralateral responses over the course of the five testing days (Day x Group: $F_{12,324}=2.41$, $p<0.05$; Simple Effect: $F_{4,78}=5.46$, $p<0.01$).

Accuracy. Intact rats were overall more accurate than lesion rats, but there was no overall difference between the lesion groups treated with saline, L-DOPA, or bromocriptine (Figure 7.17F-G; Group: $F_{3,81}=31.17$, $p<0.001$). The lesion induced deficit manifested itself when rats were required to make contralateral responses (Side x Group: $F_{3,81}=59.97$, $p<0.001$; Simple Effect: $F_{3,81}=69.06$, $p<0.001$). Restricting the analysis to the contralateral side showed a further exacerbation of the deficit in lesion rats treated with L-DOPA, but not bromocriptine (Group: $F_{3,81}=68.60$, $p<0.001$).

7.4.3.3. Effect of acute saline on LCRT task performance

At the end of the initial post-lesion testing, the effect of acute saline, L-DOPA, and bromocriptine on LCRT task performance was tested. The following section describes the effect of acute saline administration on the number of usable trials, reaction time, movement time, and accuracy in drug naive intact and lesion rats.

Usable trials. Lesion rats continued performing fewer usable trials than controls (Figure 7.18A; Group: $F_{3,21}=8.20$, $p<0.01$) but there was no difference between the usable trials made by paired and unpaired groups. While there was some variation in the usable trials made by the intact unpaired group, their number of usable trials made on the day of saline administration did not differ from other days (Day x Group: $F_{12,84}=3.44$, $p<0.001$; Effect of Day in unpaired intact group: $F_{4,18}=5.94$, $p<0.01$), suggesting that saline did not impact on the number of usable trials rats made on the LCRT task. The number of usable trials made remained similar in both five day testing cycles (Cycle: $F_{1,21}=1.13$, n.s.)

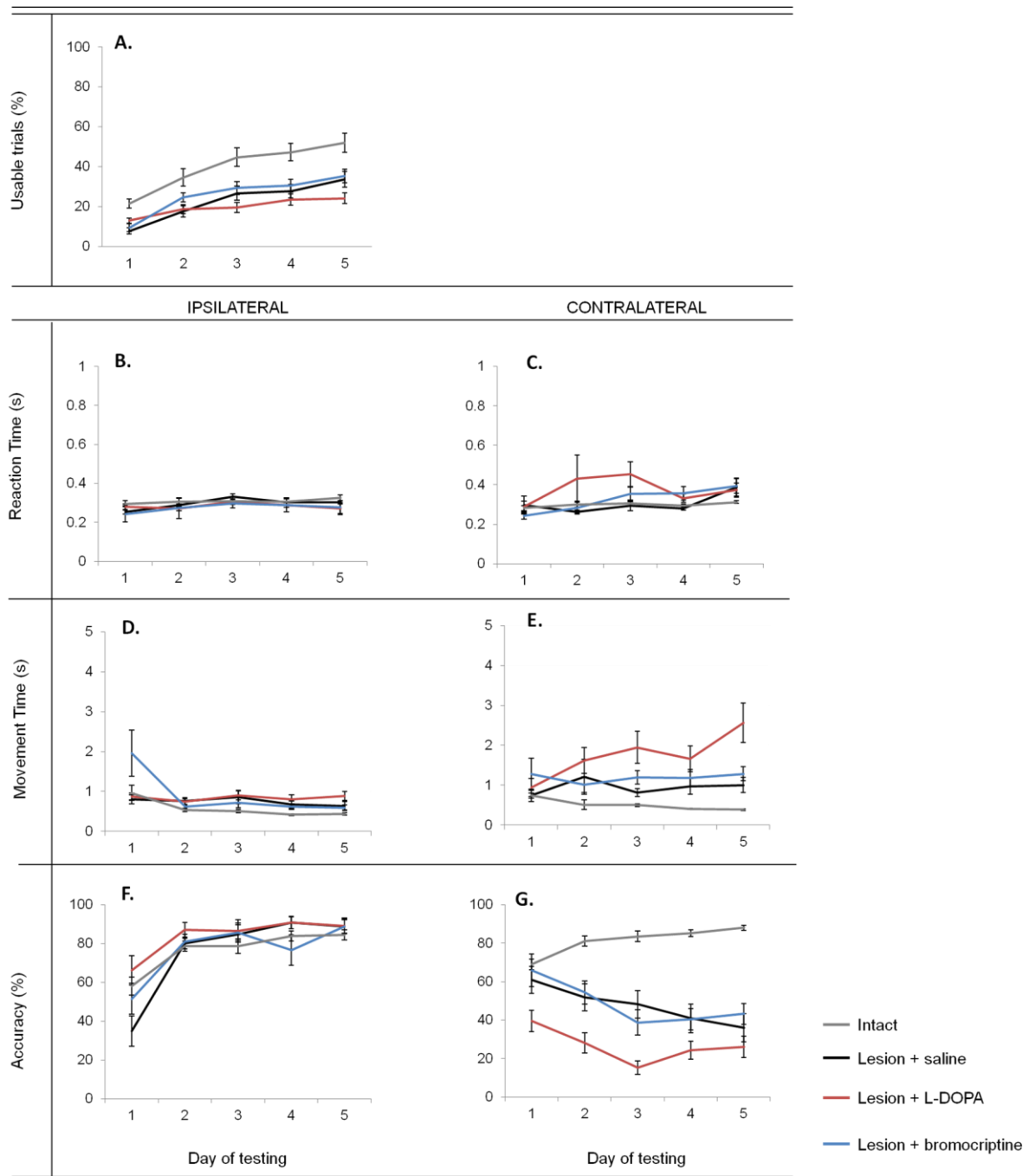


Figure 7.17. The percentage of usable trials (A), ipsilateral and contralateral reaction time (B-C), movement time (D-E), and accuracy (F-G) of the intact (n=30), lesion + saline (n=15), lesion + L-DOPA (n=25), and lesion + bromocriptine (n=15) groups. The intact groups treated with saline, L-DOPA, and bromocriptine have been pooled for clarity. The lines show the mean, and the error bars represent the standard error of the mean. BRC = bromocriptine.

SALINE CHALLENGES

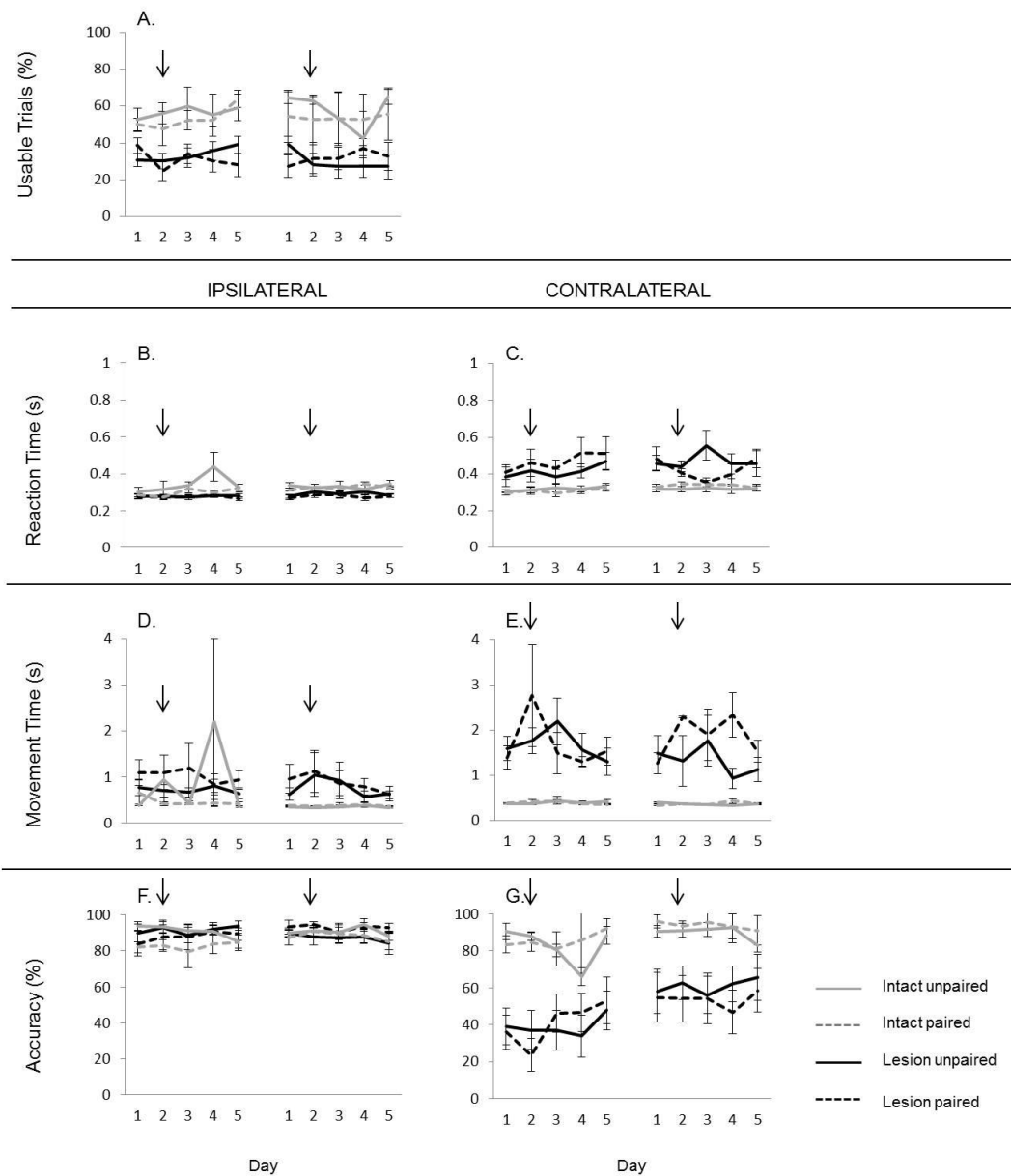


Figure 7.18. The usable trials (A), reaction time (B-C), movement time (D-E), and accuracy (F-G) of intact (paired $n=5$, unpaired $n=5$) and lesion (paired $n=7$, unpaired $n=8$) rats given acute saline prior to testing or not. The arrows indicate the day of saline administration. The lines show the mean and the error bars represent the standard error of the mean.

Reaction time. The lesion groups continued requiring longer time to respond to cues than intact controls (Figure 7.18B-C; Group: $F_{3,21}=4.12$, $p<0.05$). As expected, administering saline prior to LCRT task testing did not affect reaction times (Day x Group: $F_{12,84}=0.89$, n.s.; Day x Side x Group: $F_{4,84}=0.51$, n.s.). There was a slight difference between the ipsilateral reaction time recorded in the two five-day testing cycles. This was due to noise in the data on

the fourth day of testing and as such not related to the saline administration (Cycle x Side: $F_{1,21}=11.72$, $p<0.01$; Simple Effect of Cycle: $F_{1,21}=9.10$, $p<0.01$).

Movement time. Lesion rats continued showing longer movement times than intact controls, but as expected there was no significant difference between the paired and unpaired groups (Figure 7.18D-E; Group: $F_{3,21}=5.10$, $p<0.05$). Movement times were similar across both five-day testing cycles (Cycle: $F_{1,21}=0.30$, n.s.; Cycle x Group: $F_{3,21}=0.71$, n.s.; Cycle x Side: $F_{1,21}=3.13$, n.s.).

Accuracy. Lesion rats continued being less accurate than intact controls when responding to contralateral cues but there was no difference in the accuracy of the paired and the unpaired groups (Figure 7.18F-G; Day x Side x Group: $F_{12,84}=2.88$, $p<0.01$; Simple Effect: $\min.F_{1,21}=16.18$, $p<0.001$). There were slight differences between performance in the two five-day testing cycles (Cycle x Side x Day: $F_{4,84}=107.10$, $p<0.001$; Simple Effect of Cycle: $F_{1,21}=\min.6.35$, $p<0.05$). However, this was due to both lesion groups being more accurate in their contralateral responding during the second five day testing cycle rather than being an effect of acute saline administration.

7.4.3.4. Effect of acute L-DOPA on LCRT task performance

Usable trials. The lesion rats continued producing fewer usable trials than controls, but there was no difference between the L-DOPA paired and unpaired groups (Figure 7.19A; Group: $F_{3,30}=8.93$, $p<0.001$; Day x Group: $F_{12,120}=1.39$, n.s.). There was a slight but significant difference between the usable trials made by the intact paired rats in the first and second five day testing cycle (Cycle x Group: $F_{1,27}=3.90$, $p<0.05$; Simple Effect of Cycle: $F_{1,27}=4.24$, $p<0.05$). This was due to marginally fewer usable trials made by the intact paired group during the first five-day testing cycle.

Reaction time. Lesion rats continued showing longer reaction times than intact controls (Figure 7.19B-C; Group: $F_{3,30}=6.04$, $p<0.01$) but there was no difference between the reaction time of the paired and unpaired groups (Day x Side x Group: $F_{12,120}=1.22$, n.s.; Day x Side: $F_{4,120}=1.80$, n.s.). Lesion rats' had marginally higher reaction times in the second than first five-day testing cycle (Cycle x Group: $F_{3,30}=4.63$, $p<0.01$; Simple Effect of Cycle on the lesion groups: $F_{1,30}=\min.17.63$, $p<0.001$). However, this was not specific to a difference

L-DOPA CHALLENGES

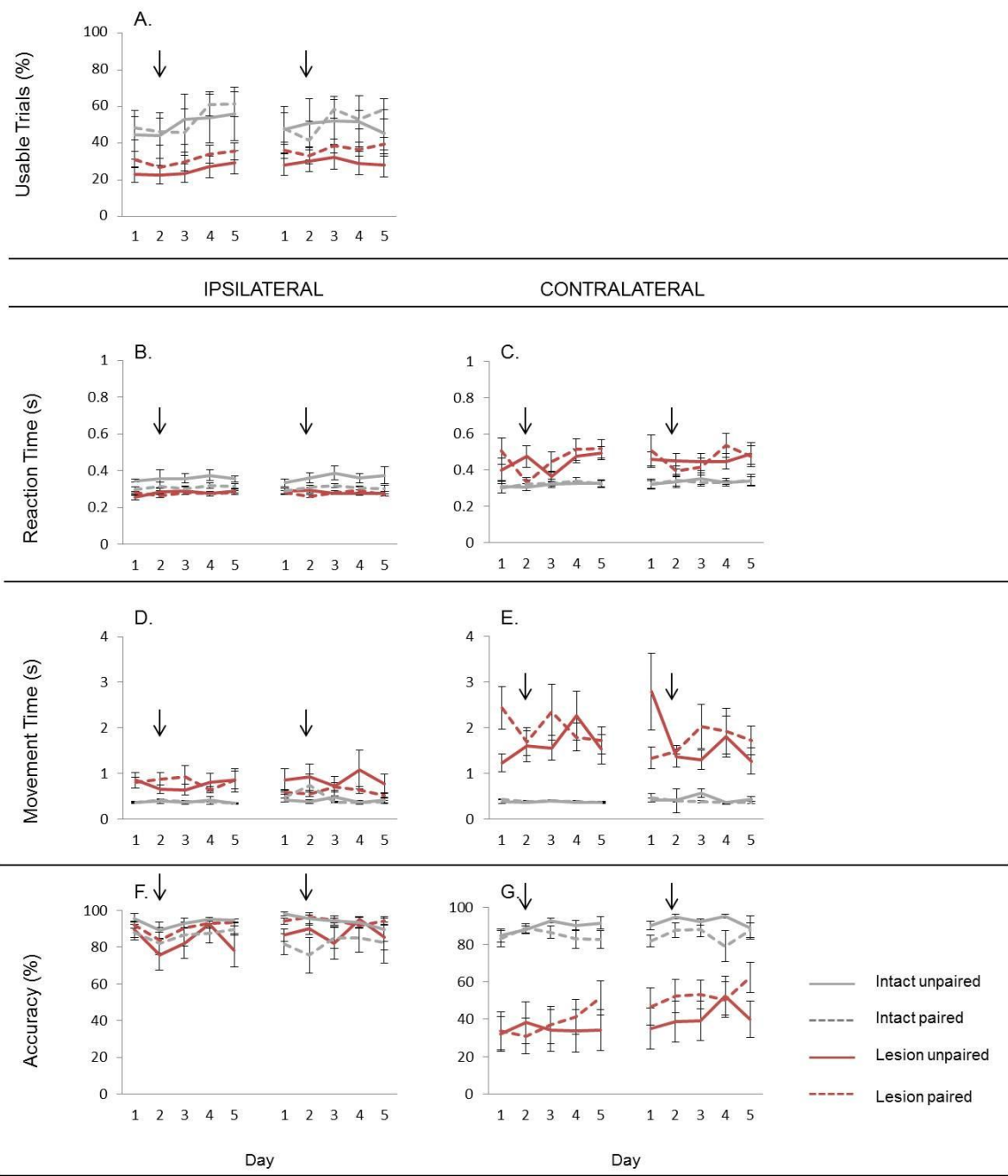


Figure 7.19. The usable trials (A), reaction time (B-C), movement time (D-E), and accuracy (F-G) of intact (paired n=5, unpaired n=4) and lesion (paired n=13, unpaired n=12) rats administered an acute L-DOPA prior to testing or not. The arrows indicate the day of L-DOPA administration. The line chart shows the mean performance of the groups and the error bars represent the standard error of the mean.

between paired and unpaired rat and as such not indicative of an effect of acute L-DOPA on reaction times.

Movement time. Lesion rats continued showing longer movement times than intact controls (Figure 7.19D-E; Group: $F_{3,30}=7.70$, $p<0.001$). Acute L-DOPA abolished the contralateral impairment in the paired lesion group on the day of administration (Day x Side x Group: $F_{4,12}=2.93$, $p<0.01$; Simple Effect: $\min.F_{3,30}=4.61$, $p<0.01$), although it is noted that the effect was marginal in the first five-day testing cycle. The recorded movement time was similar across the two five-day testing cycles (Cycle: $F_{1,30}=0.19$, n.s.; Cycle x Group: $F_{3,30}=0.86$, n.s.).

Accuracy. Lesion rats continued being less accurate than intact controls but there was no difference between paired and unpaired rats (Figure 7.19F-G; Group: $F_{3,30}=5.41$, $p<0.01$; Day x Side x Group: $F_{12, 120}=0.87$, n.s.) as would have been expected if L-DOPA alleviated the accuracy deficit. Whereas there was a difference in the accuracy of responding in the first and second five-day testing cycle, this was driven by variation in the ipsilateral accuracy data (Cycle x Side x Day: $F_{4,120}=60.52$, $p<0.001$; Simple Effect of Cycle: $F_{4,27}=105.84$, $p<0.001$) and not related to differences in the performance of paired and unpaired rats.

7.4.3.5. Effect of acute bromocriptine on LCRT task performance

Usable trials. The lesion groups continued producing fewer usable trials than controls (Figure 7.20A; Group: $F_{3,21}=10.57$, $p<0.001$). Acute bromocriptine administration caused a transient decrease in the number of usable trials made by the paired lesion group on the day of administration (Day x Group: $F_{12,84}=3.07$, $p<0.01$; Simple Effect of Day: $F_{4,18}=4.06$, $p<0.05$). There was a significant difference between the performance across the two five-day testing cycles (Cycle x Day x Group: $F_{12,64}=3.24$, $p<0.01$) driven by more usable trials being made by the paired lesion and paired intact groups on the day of bromocriptine administration in the second testing cycle (Simple Effect of Cycle: $\min.F_{1,16}= 6.35$, $p<0.05$).

Reaction time. Whilst the lesion continued impairing reaction times (Figure 7.20B-C; Group: $F_{3,21}=12.00$, $p<0.001$), the difference between the intact and the paired lesion group disappeared on the day of bromocriptine administration when the paired lesion group exhibited a temporary improvement in their reaction times (Day x Group: $F_{12,84}=2.04$, $p<0.05$; Simple Effect: $\min.F_{3,21}= 9.80$, $p<0.001$). There was a significant difference between the reaction time recorded in the first and second five-day cycle (Cycle x Side x Day: $F_{4,84}=11.93$, $p<0.001$). This was driven by marginally longer reaction times on the day of bromocriptine administration in the second, relative to the first, cycle five-day testing cycle, and a fluctuation in the ipsilateral reaction times recorded on day 4 of each cycle and

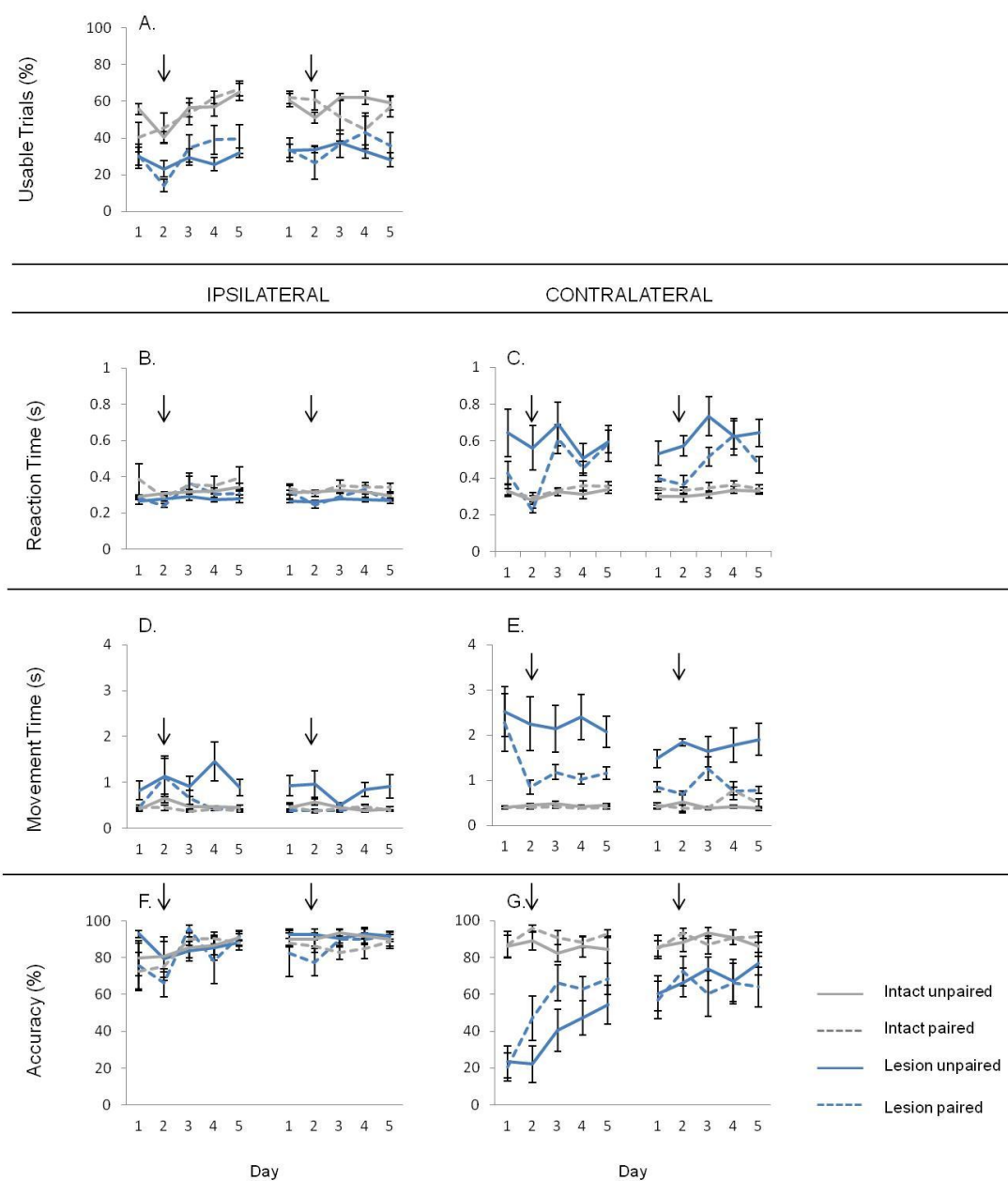


Figure 7.20. The usable trials (A), reaction time (B-C), movement time (D-E), and accuracy (F-G) of intact (paired $n=5$, unpaired $n=5$) and lesion (paired $n=7$, unpaired $n=8$) rats administered an acute L-DOPA prior to testing or not. The arrows indicate the day of bromocriptine administration. The line charts show the mean performance of the groups and the error bars represent the standard error of the mean.

contralateral reaction times on the final day of each cycle (Simple Effect of Cycle: $F_{1,21}=\text{min}.7.30$, $p<0.05$).

Movement time. Lesion rats continued exhibiting longer movement times than controls (Figure 7.20D-E; Group: $F_{3,21}=15.87$, $p<0.001$). Acute bromocriptine decreased the

time taken for the paired lesion group to make contralateral responses on the day of administration (Day x Side x Day: $F_{12,84}=4.04$, $p<0.001$; $\text{min.}F_{1,21}=5.80$, $p<0.05$). Overall, the variation in the groups' movement time was less pronounced in the second than in the first five-day testing cycle (Cycle: $F_{1,21}=4.97$, $p<0.05$.; Cycle x Group: $F_{3,21}=2.35$, n.s.).

Accuracy. The lesion rats continued making fewer accurate responses than intact controls when responding to contralateral cues (Figure 7.20F-G; Group: $F_{3,21}=4.71$, $p<0.05$; Side x Group: $F_{3,21}=4.63$, $p<0.05$; Simple Effect: $F_{3,21}=5.69$, $p<0.01$). Acute bromocriptine administration caused a gradual improvement of paired lesion rats' contralateral accuracy starting on the day of administration. In addition, there was a gradual increase in accuracy with which the unpaired group responded to contralateral cues which began the day after acute bromocriptine administration. By the second five-day testing cycle, the lesion rats' performance had reached a plateau (Day x Side x Cycle: $F_{4,84}=2.47$, $p<0.05$; Simple effect of Day: $F_{1,18}=13.32$, $p<0.001$)

7.4.4. Discussion

The experiment was conducted to replicate previous findings of a further impairment in the accuracy with which lesion rats responded to contralateral cues on the LCRT task following chronic L-DOPA treatment while also investigating the effect of chronic bromocriptine, which unlike L-DOPA, does not induce LID, on LCRT task performance. It was hypothesised that chronic L-DOPA, but not bromocriptine, would further impair lesion rats' performance on the CRT task. At the end of testing, the opportunity was also seized to test effect of acute saline, L-DOPA, and bromocriptine on LCRT task performance.

7.4.4.1. The effect of L-DOPA

The results replicated previous findings (section 7.3) of a further impairment in LCRT task performance in lesion rats chronically treated with L-DOPA, as well as showing that chronic L-DOPA treatment only affected performance of dopamine denervated rats and not performance of intact rats.

In accordance with what is already known about L-DOPA's beneficial effects on motor function (Birkmayer & Hornykiewicz, 1961), acute administration of the drug improved lesion rats' movement time. Interestingly, it did not affect accuracy which suggested it was unable to restore non-motor function measured in the LCRT task at the current dose.

7.4.4.2. The effect of bromocriptine

Contrary to what was observed following chronic L-DOPA, there was no effect of chronic bromocriptine on lesion rats' LCRT task performance. As elaborated on in the chapter discussion, the dissociate effect of chronic L-DOPA and bromocriptine is hypothesised to relate to changes in synaptic plasticity following LID onset, which is only induced by L-DOPA.

Whilst no difference was observed between the performance of paired and unpaired lesion rats prior to the acute bromocriptine challenges, both groups exhibited an increase in contralateral accuracy the days following acute bromocriptine administration. Interestingly, a closer inspection of the data (Figure 7.20G) showed that the paired lesion group's contralateral accuracy began increasing at the day of administration whilst the unpaired lesion group's contralateral accuracy only began to increase the following day. Whilst a definitive interpretation is not feasible without further investigation, the results may suggest that the bromocriptine administered to the unpaired group 2 hours after operant testing induced a behavioural change similar to that observed in the paired group that became apparent in the next testing session.

Bromocriptine has a half-life of approximately 3 h (Lieberman & Goldstein, 1985), making it unlikely that the increase in the unpaired lesion group's accuracy the day after administration was driven by an acute effect of bromocriptine. It could be hypothesised that bromocriptine induce downstream changes that affect operant behaviour. This, however, appears unlikely in the light of the current findings as lesion rats chronically treated with bromocriptine did not differ from saline treated lesion controls prior to the paired and unpaired bromocriptine administrations. It is also noted that the lesion rats treated with saline also showed higher accuracy in the second relative to the first five-day testing cycle. Based on the current data alone, conclusions on the effect of acute bromocriptine on LCRT task accuracy can therefore not be drawn

7.4.4.3. Conclusions

The data demonstrated a replicable effect of L-DOPA treatment on lesion rats' performance, wherein chronic L-DOPA impairs later contralateral accuracy on the LCRT task. The deficit was specific to lesion rats, and did not occur following chronic treatment with the clinically available D2 agonist bromocriptine which unlike L-DOPA does not induce LID. A second aim of the experiment was to test the acute effects of L-DOPA and bromocriptine on the LCRT task. It was concluded that whilst both drugs improved performance they affected different parameters. Acute L-DOPA alleviated motor impairments but was unable to

improve contralateral accuracy. Conversely, acute bromocriptine improved accuracy on the day of administration while no effect was observed on motor function. Based on the findings from this, and the previous experiment, it was hypothesised that cellular changes associated with LID onset, which was induced in lesion rats following chronic L-DOPA treatment, underlie the decreased accuracy with which lesion rats responded to contralateral cues on the LCRT task. This is further discussed in section 7.5.3.

7.5. Discussion

7.5.1. The LCRT task deficit

The LCRT task was initially developed by Carli and colleagues (1985) as a means of testing the effect of dopamine loss on sensorimotor function in rats with unilateral 6-OHDA lesions. The task allows recording of reaction and movement time, as well as the accuracy with which rats respond to visual cues presented on either the ipsilateral or contralateral side of the lesion. The lateralised nature of the task is thus suitable for rats with unilateral lesions, as it enables comparisons between responses made on the ipsilateral and contralateral sides to the lesion.

Reaction time in the LCRT task measures the time elapsed between onset of a lateralised light cue and withdrawal from the central hole, i.e. initiation of a movement in response to the cue. In addition to the motor initiation aspect the measure includes a movement element, and could be argued to also contain a level of visual attention. For example, if lesion rats were impaired relative to controls in their reaction but not motor time, the deficit could be argued to represent an attentional impairment. However, previous publications have demonstrated that the LCRT reaction time deficit observed in rats with unilateral 6-OHDA lesions depends on the direction of the movement the cue indicates (i.e. ipsilateral or contralateral to the lesion), rather than the location of the visual cue itself (Carli *et al*, 1985). Therefore, the 6-OHDA induced reaction time deficit is unlikely to reflect impaired visual attention and is instead hypothesised to be driven by a motor deficit.

In addition to reaction time, the LCRT task also records movement time, which is defined as the time elapsed between withdrawal from the central hole and completion of a correct lateralised nose poke. While the initial LCRT task publication did not report a movement time deficit in 6-OHDA lesion rats (Carli *et al*, 1987), a contralateral movement deficit was observed in later studies where the distance between the central hole and the lateralised response holes was increased (Dowd & Dunnett 2004a,b, 2005). Increased

movement times relative to healthy controls have also been observed in PD patients. For example, Sanes and colleagues (1985) demonstrated that PD patients took longer than healthy controls to move a stylus, held like a pen, between two targets. In line with preclinical data suggesting that lesion rats' LCRT movement time deficit is dependent on the distance to the response hole (Carli *et al*, 1984; Dowd & Dunnett, 2004, 2005a,b) Sanes and colleagues (1985) demonstrated a greater movement time deficit in PD patients when the distance between the targets increased. Hence, the movement time deficit is believed to reflect a lesion induced motor deficit also observed in PD.

In addition to reaction and movement times, the LCRT task measures the accuracy with which rats perform contralateral and ipsilateral responses. Preclinical data have shown decreased contralateral accuracy following unilateral 6-OHDA lesions (Dowd & Dunnett, 200, 2005a,b). However, the precise nature of the deficit has not been established. While determining the exact psychological mechanism underlying the contralateral accuracy deficit was beyond the scope of the current experiment, a discussion of potential explanations for the deficit will be provided. First, it may be hypothesized that lesion rats were unable to detect the contralateral cue because of lesion induced visual neglect. This hypothesis may, however, be rejected based on findings of Brown & Robbins (1989) who demonstrated that rats with unilateral lesions can detect and respond to contralateral cues when cues are only presented on the contralateral side and no ipsilateral option is available.

Second, it could be argued that the lesion-induced motor impairment prevented lesion rats from performing contralateral motor actions. However, this explanation may be rejected based on previously published data showing that lesion rats will make contralateral motor responses when the task is modified so as to require them to respond to light cues presented on the ipsilateral side by making contralateral responses and vice versa (Carli *et al*, 1985).

A third hypothesis is that whilst rats were able to detect contralateral light cues, they may be unable to direct motor actions in contralateral space. This is based on a hypothesis originally proposed by Brown & Robbins (1989). Brown & Robbins (1989) utilized a task where rats with intra-striatal 6-OHDA lesions, tested in nine-hole boxes, were trained to respond to light cues presented in one of two response holes. The response holes were always located on the same, i.e. ipsilateral or contralateral, side of the lesion. Brown & Robbins (1989) showed that lesion rats were not biased to either response hole when these were located ipsilateral to the lesion. However, when both response holes were located contralateral to the lesion, lesion rats were biased to responding to the nearer, more ipsilateral, response location. Interestingly, if blocking one of the contralateral response holes

so that there was only one contralateral response location available the lesion deficit disappeared. Based on this data, Brown & Robbins (1989) argued that unilateral 6-OHDA lesions induce a response related deficit where rats are unable to *direct* contralateral responses. Clinical studies have demonstrated impaired movement accuracy in PD patients which could be argued to bear resemblance to a deficit in directing movements. For example, Rand and colleagues (2000) measured movement accuracy by contrasting the time required by PD patients and healthy controls to move a stylus towards a visual target presented on a digitizer table. The task included two conditions: one emphasised the accuracy of the movement and the other did not. Rand and colleagues (2000) found that, relative to controls, PD patients' movement time increased proportionally more in the condition that emphasised accuracy. The increased movement time was especially pronounced in the final approach phase of the movement, which the authors suggested was indicative of a deficit in movement accuracy. However, while it could be hypothesised that 6-OHDA MFB lesions impair rats' ability to direct movements in contralateral space, and that this caused an overall decrease in lesion rat's contralateral responding in the LCRT task, such a hypothesis does not explain why the contralateral accuracy would manifest itself gradually. Rather, if impaired accuracy in the LCRT task was due to an inability to direct contralateral responding, the impairment would be expected to remain stable post-lesion.

A fourth hypothesis is that dopamine loss may have decreased the motor effort rats were willing to exert for a sucrose pellet reward. This is based on the finding that dopamine is implicated in cost-effort decisions. For example, Cousins & Salamone (1994) demonstrated that food restricted rats with 6-OHDA lesions to the NAcc performed an equal number of lever presses in exchange for a sucrose pellet reward on a FR5 schedule as intact controls did. However, if food pellets were available on the floor of the operant chamber during testing the number of lever presses made by lesion rats decreased as they settled for the less palatable food pellets, while no such effect was observed in intact rats (Cousins & Salamone, 1994). It is ambiguous to what extent the findings presented by Cousins & Salamone (1994) translate to the clinic. While PD is known to increase apathy, which some authors link to motivation (Czernecki *et al*, 2002), it is unclear to what extent, if at any, the decreased willingness to exert effort for a reward described in preclinical experiments (Cousins & Salamone, 1994) translates to patients' symptoms. In terms of explaining the LCRT task accuracy deficit, it should also be noted that the previously published findings of a decreased willingness to exert effort for a sucrose reward were linked to dopamine loss in the NAcc (Cousins *et al*, 1993; Cousins & Salamone, 1994). Conversely, the LCRT task accuracy deficit has been linked to

striatal dopamine loss (Carli *et al*, 1989). While MFB lesions induce dopamine loss in both the dorsal and ventral striatum, the LCRT accuracy deficit is not observed following loss of NAcc dopamine alone (Carli *et al*, 1989). Thus, while NAcc mediated motivation may have affected performance, it is unlikely to be the primary mechanism driving the contralateral accuracy deficit.

A fifth hypothesis, first presented by Dowd & Dunnett (2004) is that dopamine denervation renders the system unable to produce a dopamine reward signal. Previous publications have demonstrated a phasic dopamine peak following unexpected rewards that is transferred to the CS presentation following repeated training (Schultz, 2010; Tobler *et al*, 2003). Based on this data, it was hypothesised that 6-OHDA lesions caused a loss of the neural reward signal, and that this in turn caused extinction of contralateral responding (Dowd & Dunnett, 2004, 2007). The notion of an impaired prediction error signal following dopamine loss, although not directly measured in Dowd & Dunnett's (2004, 2005a,b) experiments, bears similarities to clinical data. For example, Schonberg and colleagues (2010) used fMRI to record PD patients' and healthy controls' neural activity while participants performed a reward based learning task. The task required participants to choose between one of two virtual slot machines. Both machines had a pseudorandomised probability of generating a win, and participants were asked to try to maximise their winnings. The positive prediction error signal observed in the dorsolateral striatum following a winning was greater in healthy controls than patients, while no difference was observed in their negative error signal following the absence of a win (Schonberg *et al*, 2010). This suggested that the dopaminergic system in PD is impaired at generating the phasic dopamine signal that is typically observed following delivery of an unexpected reward. The hypothesis presented by Dowd & Dunnett (2004) could explain not only the decrease in contralateral responding, but also the gradual decline in accuracy, which is similar to what is observed in rats undergoing extinction on the LCRT task following physical removal of sucrose pellets (Dowd & Dunnett, 2007). However, the hypothesis cannot explain why lesion rats continue responding to contralateral cues in paradigms where no ipsilateral option is available (e.g. Brown & Robbins, 1989).

While several hypotheses regarding the precise nature of the contralateral accuracy task observed in the LCRT task may be proposed, no hypothesis explains both the gradual decline in lesion rats' contralateral accuracy and the ability of lesion rats' to respond to contralateral cues when no ipsilateral option is available. It was not within the scope of the thesis project to test the psychological nature of the LCRT task deficit. However, based on

available literature, it is likely that the phenomenon is mediated by both a decrease in motivation to exert effort for a reward, and an absence or reduction in the phasic dopamine signal that typically follows delivery of a reward.

7.5.2. The effect of acute drug administration on LCRT task performance

L-DOPA and bromocriptine are both clinically available to treat the motor symptoms of PD, with the former offering greater symptomatic relief of motor deficits. As part of this chapter, the effect of acute L-DOPA and bromocriptine administration on motor (reaction and movement time) and non-motor (accuracy) parameters of the LCRT task was tested. The results showed that while L-DOPA was able to restore motor function, only bromocriptine was able to improve the accuracy with which rats responded to contralateral cues. There thus appears to be a dissociation between the neurological functions the drugs affect. Bromocriptine's ability to reinstate responding for sucrose pellets suggests that accurate LCRT task responding relies primarily on striatal D2 function, although it cannot be ruled out based on the current experiments whether other receptors which bromocriptine show affinity to were also implicated. It is possible that acute bromocriptine increased accuracy by enhancing the reinforcing effects of the reward, in line with data suggesting that antagonizing D2/3/4 receptors reduces the reinforcing effects of cocaine, as measured by a progressive ratio task (Bari & Pierce, 2005), or that acute bromocriptine enhances responding to a reinforced lever (Beninger & Ranaldi, 1992; Ranaldi & Beninger, 1995; Sutton *et al*, 2001). This would tie in with the hypothesis that the contralateral accuracy deficit was mediated by a decreased willingness to exert effort for a reward. However, considering differences in the observed effects of acute bromocriptine administration on LCRT task responding in Experiments 9 and 6, further tests are required to determine the nature of the bromocriptine mediated improvement in responding.

7.5.3. Effect of chronic drug administration on LCRT task performance

A primary aim of this thesis project was to test whether chronic administration of dopaminergic drugs affects non-motor function in lesion rats. In the current chapter, this was tested by comparing LCRT task performance of lesion rats that were drug naïve, or chronically treated with saline, bromocriptine or L-DOPA prior to LCRT task testing. Whilst chronic saline and bromocriptine did not alter the behaviour of lesion rats, chronic L-DOPA caused a further impairment of both movement time and accuracy. A main difference between the effects of chronic L-DOPA and bromocriptine on lesion rats is that only the former induces LID. It may thus be hypothesised that the difference in LCRT task

performance between lesion rats chronically treated with L-DOPA or bromocriptine is due to differences in striatal function of lesion rats that have or have not develop LID. Among other changes, chronic L-DOPA has been associated with restored ability for cortico-striatal LTP and, following LID onset, loss of the ability for depotentiation (Picconi *et al*, 2003).

Following the initial identification of a gradually emerging contralateral accuracy deficit in drug naïve lesion rats, Dowd & Dunnett (2004, 2007) hypothesised that the deficit represented extinction of contralateral responding following a lesion induced abolishment of the dopaminergic reward signal reinforcing such responses. It could thus be hypothesized that chronic L-DOPA further decreased lesion rats' accuracy by affecting either the generation, or utilization, of the dopamine reward signal. However, there was no difference in the dopamine denervation observed in lesion rats treated with saline and L-DOPA in Experiments 6 and 8 (sections 7.3 and 7.4). While not directly tested, this suggests that there was no difference in their system's ability to *generate* a phasic dopamine reward signal. Chronic L-DOPA does not decrease binding to D2 receptors (Aubert *et al*, 2005) which data in Experiments 9 and 6 (sections 7.2 and 7.3) suggest are involved in accurate LCRT task responding. Therefore, while the possibility cannot be firmly excluded based on current data, it is counterintuitive that lesion rats chronically treated with L-DOPA would be impaired relative to saline treated controls in their ability to detect a dopamine reward signal, and that the LCRT accuracy deficit was instead mediated by other factors.

While chronic L-DOPA is unlikely to have affected the generation, or utilization of, a dopamine reward signal it may have had an impact on extinction. Extinction is believed to not represent unlearning of a previous rule, but instead the learning of a new CS-US association in which the CS is no longer predictive of a reward and, as a result, the original CR is inhibited (Myers & Davis, 2002). This learning is believed to rely on synaptic plasticity, and LTP induction has been shown to be essential for e.g. fear extinction in rats (Farinelli *et al*, 2006). Whilst the data by Farinelli and colleagues (2006) was obtained from the hippocampus, it may be hypothesised that cortico-striatal LTP is implicated in extinction of striatally mediated behaviour such as LCRT task performance. Chronic L-DOPA restores the ability for cortico-striatal LTP formation in lesion rats (Picconi *et al*, 2003). It may thus be argued that, in the absence of a perceived reinforcement following a correct contralateral response, rats that had received chronic L-DOPA treatment were able to more readily acquire a new CS-US association, in which the original CR was inhibited – thereby increasing the rate with which their contralateral responding decreased. Whilst providing a potential explanation for the data presented in this chapter, the hypothesis requires further testing.

Future experiments may wish to test whether e.g. experimental inhibition of LTP formation in lesion L-DOPA treated rats abolishes the effect of chronic L-DOPA on contralateral accuracy, as would be predicted from the above hypothesis.

7.5.4. Conclusions

The data extended on previous knowledge by suggesting that contralateral accuracy on the LCRT task is primarily D2 receptor driven. The data also showed a robust effect of chronic L-DOPA on the performance of lesion rats tested off-medication. Specifically, chronic L-DOPA was associated with a more rapid decline in lesion rats' contralateral accuracy. The replicable effect of chronic L-DOPA therefore suggests that long-term treatment does not only induce adverse motor symptoms, but also affect non-motor function. Interestingly, chronic bromocriptine did not affect LCRT performance. One difference between chronic L-DOPA and chronic bromocriptine treatment is that only the former is linked to the onset of LID. Therefore, Chapter 8 further explored whether the observed L-DOPA induced deficit was linked to LID onset by comparing the LCRT task performance of lesion rats that did or did not develop LID in response to chronic L-DOPA.

8. Dissociating Between the Effect of LID onset and Chronic L-DOPA on LCRT Task Performance

Background: The results presented in Chapter 7 demonstrated exacerbation of the lesion induced accuracy deficit on the LCRT task following chronic L-DOPA treatment. Chronic L-DOPA treatment induces LID in the majority of MFB lesion rats while only a small minority remain non-dyskinetic. Based on the data in Chapter 7, it could therefore not be determined whether the LCRT task deficit observed in lesion rats chronically treated with L-DOPA was due to chronic L-DOPA exposure or LID onset.

Aim: The aim of the current chapter was to dissociate between the effects of chronic L-DOPA treatment and LID onset on lesion rats' performance on the LCRT task.

Methods: In Experiment 10, an inactive vehicle or a novel anti-dyskinetic compound was co-administered with L-DOPA to create 'lesion + L-DOPA-treated' groups that did, or did not, develop LID. Following training, lesion, and drug treatments, all rats were tested on the LCRT task. In Experiment 11, LCRT task data from experiments 9 and 10 which both included non-dyskinetic rats were pooled to allow a comparison between the performance of lesion L-DOPA treated rats that did or did not develop LID.

Results: An exacerbation of the lesion induced LCRT task accuracy deficit was apparent in L-DOPA treated rats that developed LID, while lesion rats that remained non-dyskinetic following L-DOPA treatment did not differ from saline treated lesion controls. The data thus showed that LID onset, rather than chronic L-DOPA, further impairs lesion rats' responding on the LCRT task

8.1. Introduction

The results in Chapter 7 revealed a replicable effect in which chronic L-DOPA administration caused a further decrease in the contralateral accuracy of lesion rats tested on the LCRT task. Lesion L-DOPA treated rats that develop LID differ from their non-dyskinetic counterparts in a range of parameters including increased levels of prodynorphin and FosB proteins, glutamic acid decarboxylase, upregulation of D1 receptors, and increased levels of extracellular striatal dopamine following L-DOPA administration (Cenci *et al*, 1998; Andersson *et al*, 1999; Konradi *et al*, 2004; Carta *et al*, 2006). In addition, LID has been linked to altered cortico-striatal plasticity. While chronic L-DOPA restores the otherwise lost ability for LTP formation in cortico-striatal tissue from lesion rats, the ability for depotentiation, i.e. the ability to reverse a previously potentiated response to baseline levels, is lost in tissue from rats that develop LID (Picconi *et al*, 2003).

Previous publications suggest that depotentiation affects extinction learning. For example, if depotentiation is pharmacologically induced after fear memory reactivation, it disrupts the long-term retention of the memory (Kim *et al*, 2010). Similarly, inducing depotentiation after a fear extinction trial using low-frequency stimulation decreases extinction learning, as indicated by higher levels of freezing in rats that received LFS than in controls when both groups are tested in later retention trials (Farinelli *et al*, 2006). While acknowledged that the study by Farinelli and colleagues (2006) examined the hippocampal-prefrontal cortex pathway, a role of depotentiation in extinction may be of relevance to the results presented in Chapter 7.

The majority of MFB lesion rats that are chronically treated with L-DOPA develop LID (Winkler *et al*, 2002). As previously stated, dyskinetic rats are believed to lose the ability for cortico-striatal depotentiation, whereas their non-dyskinetic counterparts retain this ability (Picconi *et al*, 2003). If the lesion induced LCRT task deficit reflects extinction as proposed by Dowd & Dunnett (2004), and extinction learning is disrupted by depotentiation induction, it could be hypothesised that lesion rats that develop LID will be less likely to experience depotentiation-related disruption of extinction learning as they are unable to exhibit this form of synaptic plasticity. This may result in greater extinction learning, and therefore a more pronounced decrease in contralateral accuracy in dyskinetic relative to non-dyskinetic rats.

In order to test the effect LID onset on lesion, L-DOPA treated rats' LCRT task performance, it was necessary to compare the performance of lesion rats that did and did not develop LID in response to chronic L-DOPA. Because few MFB lesion rats remain non-

dyskinetic following long-term L-DOPA treatment (e.g. Winkler *et al*, 2002), an anti-dyskinetic compound was used to attempt to pharmacologically produce such a group in Experiment 10. The hypothesis was also tested in Experiment 11 by pooling and comparing data from dyskinetic and non-dyskinetic lesion, L-DOPA treated rats from Experiments 9 and 10.

8.2. Experiment 10: The Effect of a Novel Anti-Dyskinetic Compound on Lesion L-DOPA Treated Rats' LCRT Task Performance

8.2.1. Introduction

Despite roughly half of all PD patients experiencing dyskinesias within 5 years of L-DOPA treatment (Nutt, 2001), there is currently no treatment available that prevents the development of LID. The clinically available NMDA antagonist Amantadine has proven able to reduce the magnitude of AIMs in patients and preclinical PD models (Sawada *et al*, 2000; Breger *et al*, 2012). Preclinical and phase II/III data also suggest that AIMs magnitude can be reduced without compromising the beneficial effects of L-DOPA on motor function using other pharmacological agents such as mGlu5 antagonists (Verhagen Metman *et al*, 1998; Johnston *et al*, 2010; Morin *et al*, 2012; Rascol *et al*, 2011), 5HT1A/B antagonists (Bézdard *et al*, 2013), and partial 5HT1A agonists (Lundblad *et al*, 2005; Eskow *et al*, 2007; Bonifati *et al*, 1994).

NMDA receptors' involvement in LID is demonstrated both by the anti-dyskinetic effects of the clinically available NMDA antagonist Amantadine (Sawada *et al*, 2010; Breger *et al*, 2012) and by findings of aberrant NMDA-dependent synaptic plasticity in tissue from lesion rats that developed LID (Picconi *et al*, 2003). Functional NMDA receptors contain NR2A and NR2B subunits, both of which are found in striatal neurons (Landwehrmyer *et al*, 1995), and which express glycine and glutamate binding sites respectively (Prybylowski & Wenthol, 2004). Among the many proteins known to interact with NR2A and NR2B subunits are the membrane associated guanylate kinases (MAGUKs). Amongst their other functions, MAGUKs are believed to stabilise the delivery of NR2B subunits to synapses, and bring NMDA receptor modulators into close proximity with the channel (Prybylowski & Wenthold, 2004). MAGUKs also interact with NR2A subunits, in particular the MAGUK protein PSD-95, which is located in the postsynaptic membrane (Kim & Sheng, 2004).

Preclinical data analysing NMDA subunit levels in the postsynaptic compartment of striatal rat tissue have demonstrated an increase in NR2A levels and a decrease in NR2B

levels in lesion rats and primates that develop LID (Gardoni *et al*, 2006; Hallet *et al*, 2006). This has led to the hypothesis that LID is caused by an increased NR2A/B ratio (Gardoni *et al*, 2012). This hypothesis is supported by preclinical data showing that administering a cell permeable TAT2B peptide to lesion, otherwise non-dyskinetic rats can decrease NR2B levels and induce acute AIMs (Gardoni *et al*, 2006). It is also supported by findings that administration of a cell permeable TAT2A peptide which is known to decouple the PSD-95/NR2A complex (Gardoni *et al*, 2006; Paille *et al*, 2010) and reduce NR2A levels in the postsynaptic compartment (Vastagh *et al*, 2012), decreases the incidence of LID in lesion rats when co-administered with L-DOPA (Gardoni *et al*, 2012). The TAT2A peptide does not, however, reduce AIMs if administered to rats that have already developed LID (Gardoni *et al*, 2012). This suggests that decoupling the PSD-95/NR2A complex and reducing TAT2A levels in the postsynaptic compartment can prevent LID onset in rats treated with L-DOPA, but is unable to reverse pathological changes underlying LID once these have occurred.

Unlike the clinically available anti-dyskinetic drug Amantadine, which suppresses the expression of dyskinesia, this TAT2A peptide has therefore been shown able to prevent LID onset by targeting molecular mechanisms associated with dyskinesia (Gardoni *et al*, 2012). To test the hypothesis that the contralateral accuracy deficit observed in lesion rats tested on the LCRT task following chronic L-DOPA treatment was caused by LID onset it was necessary to compare the performance of lesion L-DOPA treated rats that are dyskinetic and non-dyskinetic. The current experiment therefore administered the TAT2A peptide that has previously been shown to hold anti-dyskinetic effects (Gardoni *et al*, 2012) to a subgroup of lesion L-DOPA rats in order to allow for a comparison between dyskinetic and non-dyskinetic rats' LCRT task performance.

8.2.1.2. Aims

The primary aim was to compare the performance of lesion, L-DOPA treated rats that did or did not develop LID on the LCRT task.

8.2.2. Methods

8.2.2.1 Experimental design

After training on the LCRT task a subgroup of rats underwent unilateral 6-OHDA, MFB lesions. After recovery, lesion extent was assessed using the cylinder test and spontaneous rotations. Based on the results, lesion rats were allocated to one of four groups and chronically treated with saline + TAT2A; saline + SDV; L-DOPA + TAT2A; or L-DOPA + SDV. Intact rats were divided into four groups, whose treatments matched those of lesion

rats. The experimenter was blinded to the rats' treatment regimen during AIMs scoring. Following drug treatment and dyskinesia scoring, rats were food restricted and tested on the LCRT task for 5 days. Whilst testing was conducted off-medication, rats received daily injections with their respective treatments at the end of each testing day, in line with the protocol described by Gardoni and colleagues (2012) when they first reported on the TAT2A peptide's anti-dyskinetic effects.

8.2.2.2. Procedure

Eighty-nine female Lister Hooded rats were trained on the LCRT task (section 2.7.2.2) for six weeks, after which 58 rats were given unilateral 6-OHDA lesions of the MFB (section 2.2.2). Two weeks post-lesion, lesion extent was assessed using the cylinder test (section 2.4.4) and by measuring rats' spontaneous rotations in automated rotometers (section 2.4.6.3). These behavioural tests were used to balance the lesion groups. Intact and lesion rats were then chronically treated with saline+TAT2A (intact n=9, lesion n=14), saline + SDV (intact n=9, lesion n=13), L-DOPA+TAT2A (intact, n=9, lesion n=13), or L-DOPA + SDV (intact n=9, lesion n=13) for 3 weeks. In accordance with the protocol described by Gardoni and colleagues (2012), TAT2A or the inactive control peptide (SDV), which lacked the relevant NR2A interaction domain (Gardoni *et al*, 2012), were injected i.p. 20 min prior to L-DOPA administration at a volume of 1 ml/kg and a concentration of 3 nmol/kg during the first week of treatment. The TAT2A and SDV dose was increased to 6 nmol/kg two weeks into treatment when it became apparent that there was no effect of the TAT2A peptide on AIM scores. L-DOPA was administered s.c. at a concentration of 10 mg/kg, with 15 mg/kg benserazide, at a volume of 1 ml/kg throughout the testing period. The dyskinetic effect of L-DOPA + TAT2A/SDV was assessed by scoring animals' AIMs three times weekly, with the first scoring taking place 20 min after L-DOPA administration, i.e. 40 min after TAT2A/SDV administration. The TAT2A and SDV were prepared by a second person who dispensed the drugs into vials that were numbered but did not contain the name of the drug on them, so as to ensure that the thesis author was blinded to the content of the prepared drug vials and group allocation during AIMs scoring.

Following chronic treatment, rats were food restricted for 1 week, and thereafter tested on the LCRT task for five days. Although rats were tested off-medication, they received daily injections of their respective drugs at the end of the daily testing session to ensure consistency with the dosing regimen reported by Gardoni and colleagues (2012). At the end of LCRT task testing, rats were euthanized by an overdose of Euthatal, the striatum

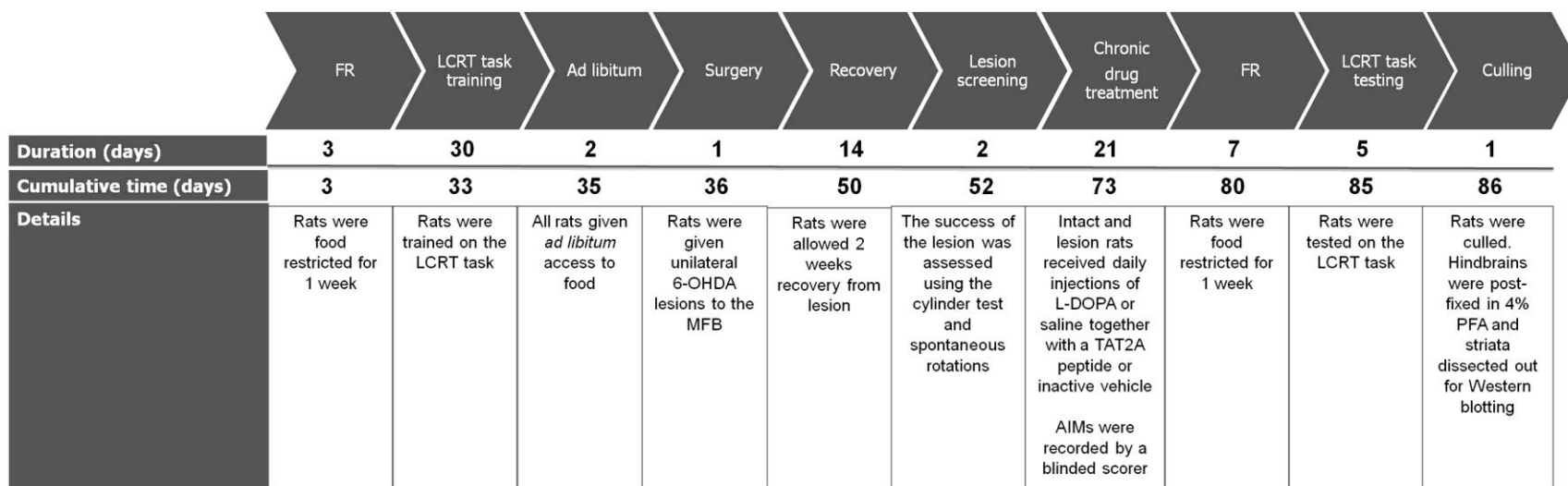


Figure 8.6. A graphical representation of the experimental design of Experiment 10. AIMs=Abnormal involuntary movements, FR=Food restriction, LCRT=Lateralised Choice Reaction Time task, MFB=Medial forebrain bundle, PFA=Paraformaldehyde

dissected fresh and immediately placed on dry ice (2.9), and the hindbrain fixed in 4% PFA and used for TH staining of the nigra (2.11). Striatal samples were sent to Dr. Fabrizio Gardoni's (Department of Pharmacology, Milan University, Italy) lab where the tissue was homogenised and Western blots run to detect NR2A and NR2B levels as described in section 2.12. It should be noted that data that were returned from the collaborators showed the data from experimental groups expressed as a percentage of the intact group treated with saline and SDV, and that no raw data was available. Therefore, data from the intact group treated with saline and SDV was not included in the statistical analysis presented in the results section. Furthermore, the collaborators were sent striatal tissue from nine lesion, non-dyskinetic rats that had been co-treated with either the TAT2A or SDV peptide, and of which it was discovered after cell counting that not all met the criteria for having a full lesion

($\geq 90\%$ SNc dopamine loss). However, only data from four of these samples were provided, and due to missing lab notes in the collaborator's lab it was not possible to determine what tissue samples that had been used in the Western blot. Because it could not be determined whether the data that was labelled as coming from non-dyskinetic rats (i) came from rats with a similar SNc loss as the dyskinetic rats included in the analysis, or (ii) came from four non-dyskinetic rats that had received the same TAT2A/SDV treatment, and (iii) because of the large variability within the non-dyskinetic sample, it was not appropriate to present this data as coming from one experimental group. Therefore, this group was omitted from the analysis presented in the results section.

8.2.2.3. Statistical analysis

Dopamine denervation data were analysed in two separate analyses. First, the raw optical density measures from the ipsilateral versus contralateral striatum were compared using a t-test to confirm that the lesion had reduced TH+ staining in the lesion striata. Second, an ANOVA was used to test whether the experimental groups differed in the percentage loss of ipsilateral striatal TH+ immunoreactivity induced by the lesion. The ANOVA used included Region as a within-subject variable and Group as a between-subject variable.

Spontaneous rotation data were analysed using a one-way ANOVA. The cylinder test data were not normally distributed and therefore analysed using a Kruskal-Wallis test. Western blot data was only normally distributed for the NR2B levels. Therefore, NR2B levels were analysed using a one-way ANOVA whereas the NR2A levels and the NR2A/NR2B ratio were analysed using a Kruskal-Wallis test.

AIMs scores were analysed using a repeated measures ANOVA with cumulative scores from each of the six testing sessions included in the analysis. Two analyses were conducted: one analysis using the global AIMs scores as had been done in previous experiments presented in this thesis, and a second analysis only including the severity scores as presented in the article by Gardoni *et al* (2012).

LCRT task performance was measured using a repeated measures ANOVA. Significant between-group differences were analysed using a Scheffe post-hoc test and significant interactions analysed using tests of simple effects.

8.2.3. Results

8.2.3.1. Effect of lesion and drug treatments

The lesion induced a significant loss of dopaminergic cell bodies in the lesion SN (Figure 8.2A; $t_{57}=21.81$, $p<0.001$) as well as the lesion VTA (Figure 8.2B; $t_{57}=15.39$, $p<0.001$). A

further analysis demonstrated that the percentage loss of ipsilateral dopaminergic cell bodies was greater in the SN than in the VTA (Region: $F_{1,54}=12.79$, $p<0.01$) but that the extent of dopamine denervation was similar between the experimental groups (Group: $F_{3,54}=2.09$, n.s.; Region x Group: 0.50, n.s.).

Reflective of their lesions, all lesion groups showed an ipsilateral bias in their performance on the cylinder and spontaneous rotations tests (Figure 8.2C-D). The ipsilateral bias exhibited was similar between the four lesion groups (Cylinder test: $H=1.86$, $df=3$, n.s.; Spontaneous rotations: $F_{3,54}=1.62$, n.s.)

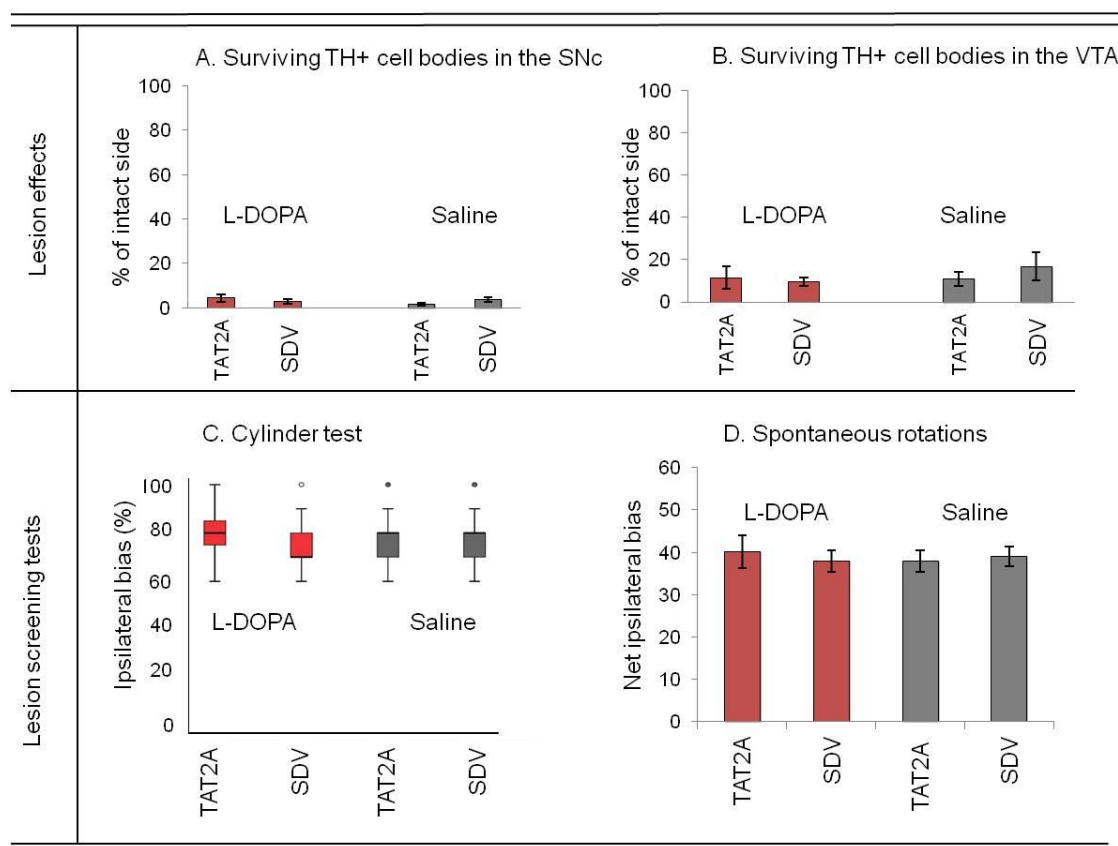


Figure 8.2. The top panel shows surviving dopaminergic cell bodies in the lesion SNc (A) and VTA (B), expressed as a percentage of the intact side, in lesion rats subsequently treated with L-DOPA (red bars) or saline (grey bars) together with the TAT2A peptide or the inactive SDV vehicle. The bottom panel shows the ipsilateral bias on the cylinder test (C) and the net ipsilateral bias on the spontaneous rotations test (D). The bar charts (A, B, D) show the mean and the error bars the standard error of the mean. The boxplot (C) shows the median and interquartile range, with circles representing outliers. SNc=Substantia nigra pars compacta, TH=Tyrosine hydroxylase, VTA=Ventral tegmental are

Despite differences in the median scores, the groups' NR2A and PSD-95 coimmunoprecipitation levels did not differ significantly from each other (Figure 8.3A; $H=6.57$, $df=6$, n.s.). NR2B and PSD-95 coimmunoprecipitation levels were higher in lesion rats treated with saline + SDV than in lesion rats treated with L-DOPA + SDV (Figure 8.3B; $F_{6,21}=3.81$, $p<0.05$) suggesting that these levels were decreased by L-DOPA treatment. Conversely, there was no difference in the NR2B and PSD-95 coimmunoprecipitation levels of lesion rats treated with saline + TAT or L-DOPA + TAT, suggesting that the TAT2A peptide prevented the L-DOPA induced increase in NR2B levels. Despite differences in the median scores, the NR2A/NR2B ratio did not differ significantly between the groups ($H=4.65$, $df=6$, n.s.). Visual inspection of the raw NR2A data and NR2A/NR2B ratio scores suggested that the lack of a significant effect was due to the large variation in the scores coupled with a small sample size ($n=4$).

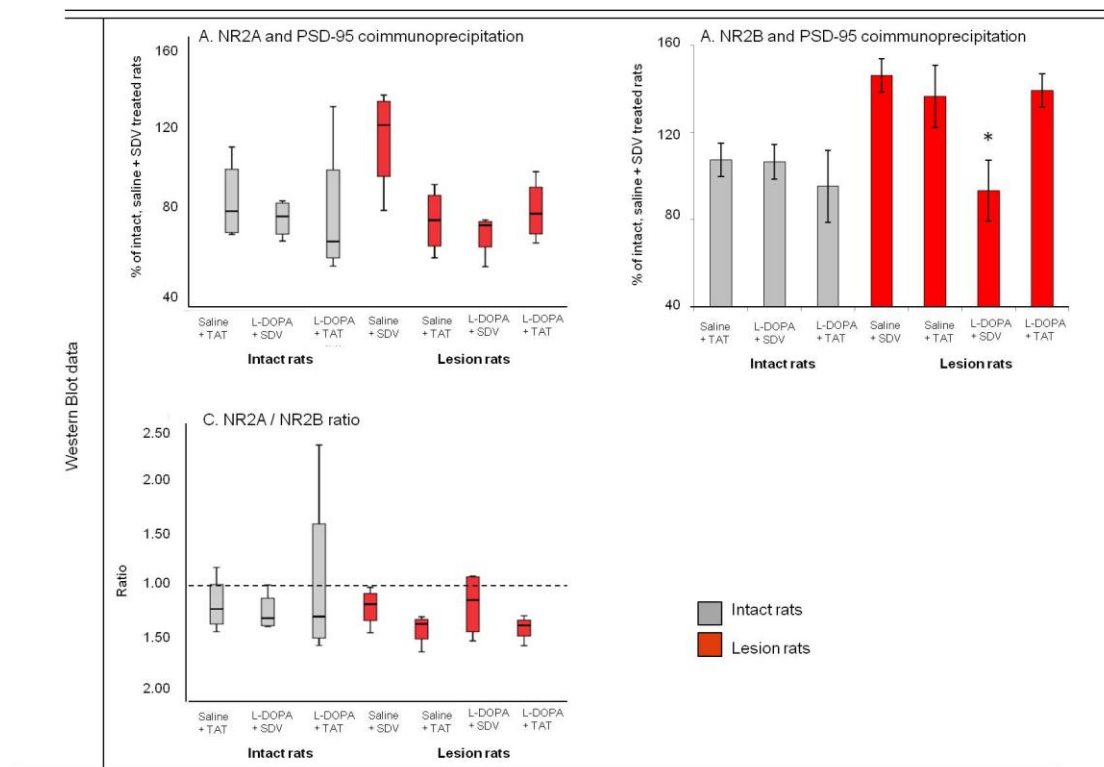


Figure 8.3. Western blot data showing NR2A (A), and NR2B levels (B), as well as the NR2A/NR2B ratio (C) in ipsilateral striatal tissue samples from intact (grey bars) and lesion (red bars) rats that were chronically treated with either saline or L-DOPA in conjunction with the TAT2A peptide or an inactive vehicle (SDV). All data are shown as percentage of the intact group treated with saline and SVD. The boxplots (A,C) show the median and interquartile ranges, while the barchart (B) shows the mean and the standard error of the mean. All $n=4$. * $p<0.05$ (versus lesion rats treated with saline + SDV)

Chronic L-DOPA induced stable AIMs in all lesion groups (Figure 8.4B), with no difference observed between rats that were co-treated with the inactive SDV vehicle or the TAT2A peptide. The absence of a significant effect of the TAT2A peptide was true both when analysing the global AIMs (Figure 8.4A-B; Group: $F_{1,27}=0.02$, n.s.; Scoring session: $F_{5,135}=12.39$, $p<0.001$; Scoring session x Group: $F_{5,135}=0.55$, n.s.) and when only including AIMs severity scores (Figure 8.4C-D; Group: $F_{1,27}=0.02$, n.s.; Scoring session: $F_{5,135}=12.64$, n.s.; Scoring session x Group: $F_{5,135}=1.07$, n.s.), which is how the AIMs scores were analysed in the original paper showing an anti-dyskinetic effect of the TAT2A peptide (Gardoni *et al*, 2012).

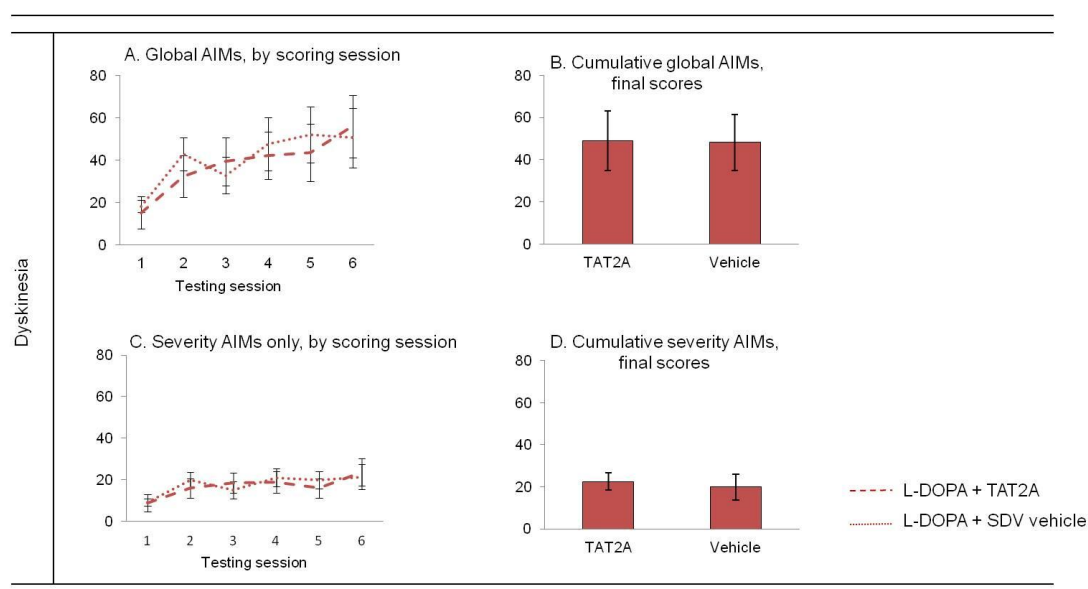


Figure 8.4. The global (top panel) and severity (bottom panel) AIMs scores recorded during each scoring session (left panel) and at the final scoring session (right panel) in lesion rats that were administered a TAT2A peptide (dotted line) or an inactive vehicle (full line) together with L-DOPA. The bar and line charts show the mean, and the error bars show the standard error of the mean. AIMs=Abnormal involuntary movements.

8.2.3.2. Post-lesion performance on the LCRT task

The data did not reveal a significant effect of the TAT2A peptide on AIMs scores (8.2.4.1.), nor was there an effect of the peptide on any of the LCRT task parameters (Appendix II). Because there was no difference between the TAT and SDV treated groups' LCRT task performance, and because the aim of the experiment was to test the effect of LID onset rather than TAT2A treatment on lesion rats' LCRT task performance, the TAT2A and SDV groups

were pooled. This led to four groups being included in the final analysis: intact + saline; intact + L-DOPA; lesion + saline; and lesion + L-DOPA.

Usable Trials. Lesion rats made fewer overall usable trials than intact rats, with a further decrease observed in lesion rats treated with L-DOPA (Figure 8.5A; Group: $F_{3,85}=3.85$, $p<0.001$). The intact rats showed an increase in the number of usable trials they made over the course of testing, whereas a slight decrease was observed in lesion rats treated with L-DOPA (Day x Group: $F_{12,340}=12.75$, $p<0.001$; Simple Effect: min. $F_{3,82}=2.61$, $p<0.05$).

Reaction Time. An initial analysis including both ipsilateral and contralateral performance was conducted. In this analysis, both of the lesion, but neither of the intact, groups showed longer reaction times when responding to contralateral than ipsilateral cues (Figure 8.5B-C; Side x Group: $F_{3,85}=9.97$, $p<0.01$; Simple Effect of Group on Side: $F_{3,85}=6.10$, $p<0.001$). However, while there was a trend toward an overall group difference this did not reach significance (Group: $F_{3,85}=2.64$, n.s.). Restricting the analysis to the contralateral side, where the lesion deficit manifested itself, confirmed that lesion rats showed longer contralateral reaction times than controls but did not reveal a significant difference between lesion rats treated with saline or L-DOPA (Figure 8.5C; Group: $F_{3,85}=6.10$, $p<0.01$). The lesion deficit manifested itself gradually, causing the lesion rats to only differ significantly from intact controls on days 3 to 5 of testing (Day: $F_{4,340}=18.57$, $p<0.001$; Day x Group: $F_{12,340}=4.15$, $p<0.001$; Simple Effect: min. $F_{3,85}=2.74$, $p<0.05$)

Movement Time. Both of the lesion, but neither of the intact, groups showed longer movement times when responding to contralateral than ipsilateral cues (Figure 8.5D-E; Side x Group: $F_{3,85}=2.88$, $p<0.05$; Simple Effect of Side: $F_{1,85}=8.87$, $p<0.01$). The difference in ipsilateral and contralateral movement times manifested itself gradually (Side x Day: $F_{4,340}=2.51$, $p<0.05$; Simple Effect of Day: $F_{4,82}=5.76$, $p<0.001$). In addition to the lesion deficit there was a further effect of L-DOPA treatment, with lesion rats treated with L-DOPA exhibiting longer movement time than lesion rats treated with saline (Group: $F_{3,85}=29.96$, $p<0.001$).

Accuracy. Lesion rats were less accurate than intact controls when responding to cues presented on their contralateral, but not ipsilateral, side (Figure 8.5G-H; Side x Group: $F_{3,85}=49.34$, $p<0.001$; Simple Effect of). The deficit manifested itself gradually, with lesion

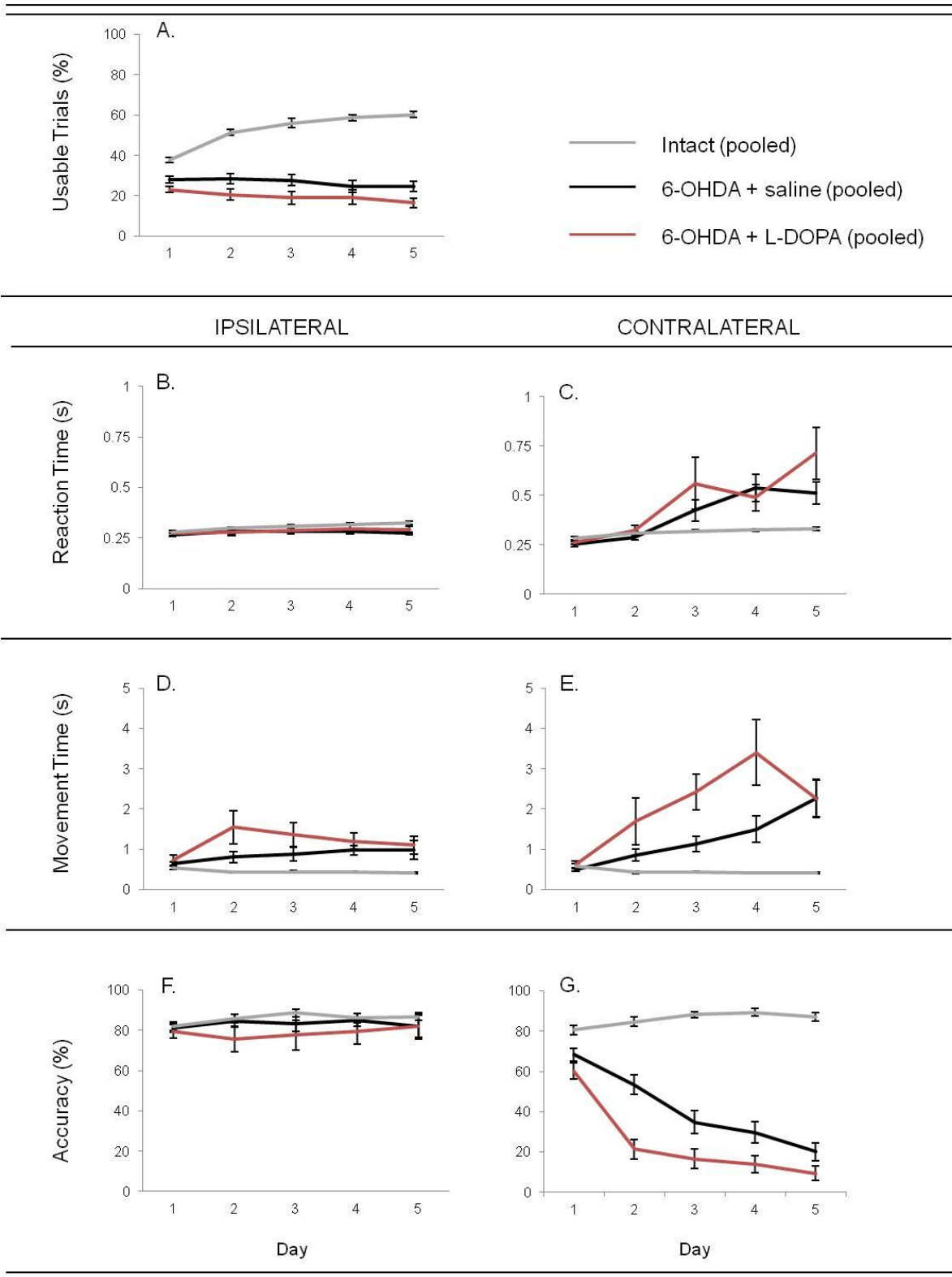


Figure 8.5. The post-lesion percentage of usable trials (A), together with the ipsilateral and contralateral reaction time (B-C), movement time (D-E), and accuracy (F-G) of intact (n=36), lesion saline treated (n=27), and lesion L-DOPA treated (n=26) rats tested on the lateralised choice reaction time task. The line chart shows the mean response on each testing day and the error bars show the standard error of the mean.

but not intact, rats showing a gradual decline in their contralateral accuracy (Side x Day x Group: $F_{12,340}=6.51$, $p<0.001$; Simple Effect of Day: $\min.F_{4,82}=24.93$, $p<0.001$). As described previously in this thesis, lesion rats that had received chronic L-DOPA treatment were less accurate than lesion rats treated with saline (Group: $F_{3,85}=64.56$, $p<0.001$).

8.2.4. Discussion

The experiment used a novel anti-dyskinetic compound (TAT2A; Gardoni *et al*, 2012) to produce a lesion L-DOPA treated group that did not develop LID, with the aim of dissociating between the effects of chronic L-DOPA versus LID onset on LCRT task performance. Unlike the previous publication by Gardoni and colleagues (2012), the current data did not demonstrate an anti-dyskinetic effect of the TAT2A peptide. It did, however, demonstrate a decrease in striatal NR2B levels in lesion, dyskinetic rats relative to lesion, saline treated rats. This is in line with what has previously been reported (Gardoni *et al*, 2006) and suggest that the peptide may have exerted a molecular effect in the current experiment. Unfortunately, to fully conclude whether or not the molecular changes observed in the dyskinetic rats aligned with previously published data it would have been necessary to compare this data with data from a lesion, L-DOPA treated non-dyskinetic cohort. Tissue sample from nine non-dyskinetic rats, two of which were not included in the behavioural analysis of the LCRT task data as their SNc denervation was less than the pre-determined 90% cut off (88.7% and 74.4% SNc loss respectively), were sent to the collaborators who conducted the Western blot analysis. These non-dyskinetic rats had been treated with either L-DOPA + SDV or L-DOPA + TAT. However, the Western blot data received from the collaborator only included a subsample of these non-dyskinetic group ($n=4$) and, due to missing lab notes in the collaborator's lab, it was not possible to determine what individual tissue samples that Western Blot data returned from the collaborators corresponded to. Because of the lack of knowledge of the lesion extent and previous drug treatment of the non-dyskinetic rats from which the Western blot data was obtained, and the considerable variation in the same Western blot data, it was not appropriate to pool these data and treat it as one homogenous non-dyskinetic group. Hence, a thorough analysis of the effect of LID and the TAT2A peptide on NR2A levels could not be achieved. Therefore, the presented data was not robust enough to either support or challenge the validity of the previously reported link between striatal postsynaptic NR2A levels and LID (Gardoni *et al*, 2012). The reason for the large variation in the Western blot data is difficult to determine as the tissue was homogenised, the Western blot run, and the optical density of the bands measured by

researchers in Dr. Fabrizio Gardoni's lab. It is possible that the variance in the data reflect natural variation between individual rats, and that a greater sample size would be required to test the hypothesis that NR2A levels are linked to LID.

While the Western blot data were not sufficiently robust to determine whether or not there was a molecular effect of the TAT2A peptide, it was evident from the AIMs scores recorded by a scorer who was blinded to the rats' treatment that the peptide did not produce an anti-dyskinetic effect in the current experiment. A key difference between the experiments reported in this thesis project and the data previously published by Gardoni *et al* (2012) is the proportion of lesion rats that remained non-dyskinetic following chronic L-DOPA treatment. While Gardoni and colleagues (2012) reported that 40% of their lesion, L-DOPA treated rats that did not receive an anti-dyskinetic drug remained non-dyskinetic, only 10% of rats with complete MFB lesions in the current experiment remained non-dyskinetic. The latter percentage of non-dyskinetic rats is similar to other in-house dyskinesia studies. The difference in rats' LID susceptibility may relate to genetic differences, as the rats in this thesis project and Gardoni and colleagues (2012) experiment were obtained from different breeding centres, or one or several of the methodological differences between the experiments discussed below.

One difference was the gender of the rats included in the studies; Gardoni *et al* (2012) used male rats, while the current experiment employed female rats. This decision was made to ensure consistency with previous experiments using the LCRT task (Chapter 7; Carli *et al*, 1985; Dowd & Dunnett, 2004, 2005a,b). However, it should also be noted that while Gardoni and colleagues used male rats, the dyskinesia scoring protocol employed in this, as well as in Gardoni *et al*'s (2012), experiment was developed using female rats (Winkler *et al*, 2002).

A second difference between the studies is the rat strain used; whereas Gardoni *et al* (2012) used Wistar rats, the current experiment used Lister Hooded rats. Lister Hooded rats were preferred as their superior vision relative to albino strains (Prusky *et al*, 2002) made them more suitable for testing on the LCRT task which requires rats to respond to visual cues. While Experiment 1 showed similar development of LID in an albino strain and Lister Hooded rats, it is not known whether rat strains differ in their response to the TAT2A peptide. Previously published data have reported strain differences in the binding to striatal NMDA receptors (Martin *et al*, 2003). Therefore, the possibility that Lister Hooded and Sprague Dawley rats differ in their NR2A subunits levels, which the TAT2A peptide targeted (Gardoni *et al*, 2012), and that this could have affected their response to the peptide treatment cannot be excluded. To test whether there are strain differences in NR2 subunits, it would be

necessary to compare baseline NR2A and NR2B levels in striatal tissue from Lister Hooded and Sprague Dawley rats, something which was beyond the scope of this experiment. However, if rat strain differences did account for the different findings, it could be queried whether the anti-dyskinetic properties of the TAT2A peptide are sufficiently robust to translate to the clinic.

A third difference between this and Gardoni *et al*'s (2012) experiment is the long period of operant training prior to lesion and drug treatment the rats in the current experiment underwent. It was suggested by the author of the original TAT2A paper (Gardoni, verbal communication) that motor training may have affected baseline NR2A/B levels which could in turn have affected the later impact of the peptide. The view is partly supported by data showing that e.g. visual stimulation increases NR2A levels in the rat visual cortex (Quinlan *et al*, 1999), suggesting that environmental exposure may affect the expression of the NR2 subunits.

It is also worthwhile to note that the operant training/testing necessitated animals to be food restricted. Previously published data have suggested that *in utero* food restriction enhances later hippocampal LTD induction in intact Sprague Dawley rats (Titterness & Christie, 2008). Similarly, food restricting adult Sprague Dawley rats has been found to enhance LTD in the motor cortex (Cohen & Castro-Alamancos, 2005). An effect of food restriction on synaptic plasticity, which has previously been linked to LID (Picconi *et al*, 2003) may therefore have impacted on LID development.

There was also a difference in the dose of benserazide used. Benserazide inhibits DOPA decarboxylase, which converts L-DOPA to dopamine, and is co-administered with L-DOPA to prevent the latter from being decarboxylated in the periphery. By reducing the amount of L-DOPA that is decarboxylated in the periphery, benserazide reduces adverse side effects such as nausea and vomiting, but also crucially increases the amount of L-DOPA that enters the brain (Da Prada *et al*, 1984). Whilst Gardoni *et al* (2012) co-administered rats 6 mg/kg benserazide, all experiments in this thesis project used a benserazide dose of 15 mg/kg. Hence, the higher benserazide dose used here may have increased the amount of L-DOPA entering the brain. Higher L-DOPA doses induce greater AIMs than lower drug doses (Lindgren *et al*, 2007) and the risk of LID onset may be reduced if lowering the administered L-DOPA dose (Putterman *et al*, 2007). Thus, it could be hypothesised that the higher benserazide dose may have increased the risk of LID development in this, relative to Gardoni and colleagues' (2012), experiment.

Despite differences in experimental designs, it is worth noting that the current experiment held several advantages over the original publication. First, there was an overall higher success rate in MFB lesions (45% in Gardoni *et al*, 2012, compared to 85% in the current experiment). Whilst both the current experiment and that reported by Gardoni *et al* (2012) ultimately only included lesion rats with near complete dopamine denervation, the difference in success rates may suggest more consistent lesions in the current experiment. Furthermore, whilst Gardoni *et al* (2012) used apomorphine induced rotations to assess lesion success the current study used non-pharmacological behavioural tests, thereby avoiding the potentially confounding effect of exposing rats to dopaminergic drugs prior to L-DOPA and TAT2A treatment. During AIMs scoring, Gardoni *et al* (2012) only measured the severity of dyskinetic movements whilst the current experiment also included the duration of AIMs thereby making the overall AIMs score more sensitive. Unlike what was reported in Gardoni and colleagues' publication (2012), the design in this experiment also ensured that the experimenter was blinded to the rats' treatment during AIMs scoring, thereby minimising experimenter bias.

It is worth noting that while the reasons for the diverging results are unclear, the primary aim of the current experiment was not to test the efficacy of the TAT2A peptide *per se* but to use it as a tool to create the non-dyskinetic lesion L-DOPA treated group needed to test the hypothesis that the previously observed exacerbation of the LCRT deficit observed in lesion rats treated with L-DOPA was related to LID onset. While the lack of an anti-dyskinetic effect of the TAT2A peptide prevented testing of the hypothesis the experiment replicated the L-DOPA induced LCRT deficit in lesion rats, thus demonstrating the robustness of the effect. It also extended our understanding of the phenomenon by demonstrating that the L-DOPA induced deficit was present following overnight, as opposed to long-term, L-DOPA withdrawal. The latter was valuable in demonstrating that the L-DOPA effect was present in a situation more closely resembling the clinically defined "off" phase (12 h post last L-DOPA dose; e.g. Cools *et al*, 2010). Finally, while the TAT2A peptide was not shown to hold anti-dyskinetic properties in the current setting, the high number of lesion L-DOPA rats included in the experiment provided a small sample of non-dyskinetic rats that could be used in the analysis described in Experiment 11.

8.3. Experiment 11: Analysis of Pooled Behavioural Data from Dyskinetic and Non-Dyskinetic Rats

8.3.1. Introduction

To test the hypothesis that the LCRT deficit was linked to LID onset, rather than chronic L-DOPA, the current analysis pooled behavioural data from dyskinetic and non-dyskinetic rats that had been used in Experiments 9 and 10. By pooling the data, it was possible to generate a lesion L-DOPA treated non-dyskinetic group with a sufficiently large n to conduct statistical analysis on. As previous experiments had found a selective effect of L-DOPA treatment on contralateral movement time and accuracy, the analysis was restricted to these parameters.

Considering the large difference in the magnitude of AIMs observed in the dyskinetic and non-dyskinetic cohort, a demonstrated difference between the two groups' LCRT task performance could be hypothesised to be linked to differences in AIMs magnitude rather than LID onset. Therefore, a second analysis was conducted in which lesion L-DOPA treated rats' AIMs scores were divided into four quartiles, and the movement times and accuracy of these four lesion L-DOPA treated groups were compared.

8.3.1.2. Aims

The primary aim was to test the hypothesis that there was a dissociation between dyskinetic and non-dyskinetic rats' performance on the LCRT task. To exclude the possibility that a difference in the two cohorts' LCRT task performance was linked to difference in AIMs magnitude rather than LID onset, the current section also tested whether AIMs magnitude affected rats' performance.

8.3.2. Methods

Pooling of the data from Experiments 9 and 10 resulted in the following groups: intact controls ($n=56$), lesion + saline ($n=42$), lesion + L-DOPA with dyskinesia ($n=42$), and lesion + L-DOPA without dyskinesia ($n=9$). A rat was defined as dyskinetic if she exhibited severity AIMs >1 on the orolingual, axial, or forelimb AIMs subtypes at any one scoring point. This classification is in line with criteria used in previous rat dyskinesia studies (Lindgren *et al*, 2010) and has been shown to distinguish between rats with high and low striatal levels of dyskinesia markers such as phosphorylated ERK1/2 (Westin *et al*, 2007), FosB, and PDyn mRNA (Andersson *et al*, 1999). In addition, following acute L-DOPA administration, rats classified as non-dyskinetic using this cut-off have been shown to have lower levels of

extracellular DOPA in the SNc and striatum than dyskinetic controls (Lindgren *et al*, 2010), and, unlike dyskinetic rats, do not show an angiogenic response to L-DOPA treatment (Westin *et al*, 2006).

A second analysis was conducted to test whether LCRT task performance was affected by the magnitude of AIMs, as opposed to the onset of LID. In the absence of published criteria for how to reliably divide dyskinetic rats into separate subgroups based on AIMs magnitude, and without obvious groupings of the rats' AIMs scores, a mathematical approach was taken where rats were divided into four quartiles based on their AIMs scores.

8.3.2.1. Statistical analysis

Dopamine denervation was analysed using two separate analyses. First, to confirm the presence of a lesion, a t-test was used to compare the raw number of cell bodies that were present in the lesion and intact SN or VTA. Second, the percentage of dopaminergic cell bodies in the lesion SN and VTA (relative to the intact side) were analysed in an ANOVA with Region as within-subject variable and Group as a between-factor variable. Spontaneous rotations were analysed using a one-way ANOVA. Ipsilateral bias on the cylinder test was analysed using a Kruskal-Wallis test. LCRT task performance was analysed using a repeated measures ANOVA with Day and Side as within-subject variables and Group as within-subject variables. Significant between-subject differences were analysed using a Scheffe post-hoc test and significant interactions analysed using a test of simple effects.

8.3.3. Results

8.3.3.1. Dyskinetic versus non-dyskinetic rats

Lesion effects. The lesion induced a significant loss of dopaminergic cell bodies in the ipsilateral SNc (Figure 8.4A; $t_{92}=-29.74$, $p<0.001$) and VTA ($t_{92}=-33.83$, $p<0.001$). A further analysis was conducted to compare the percentage SNc and VTA denervation induced in the lesion groups. This analysis confirmed that there was a greater loss of dopaminergic cell bodies in the SNc than in the VTA (Region: $F_{1,90}=28.62$, $p<0.001$) but that the extent of the dopamine loss was similar across the lesion groups (Group: $F_{2,90}=0.13$, n.s.; Region x Group: $F_{2,90}=0.26$, n.s.).

The success of the lesions was also reflected in an ipsilateral bias in rats' spontaneous rotations. Interestingly, the spontaneous rotational bias was less pronounced in the L-DOPA treated non-dyskinetic group than in the other lesion groups (Figure 8.4B; $F_{2,91}=10.23$,

$p < 0.01$). In addition, all lesion rats showed a pronounced ipsilateral bias in the cylinder test which was equal for all groups (Figure 8.4C; $H = 2.56$, $df = 2$, n.s.).

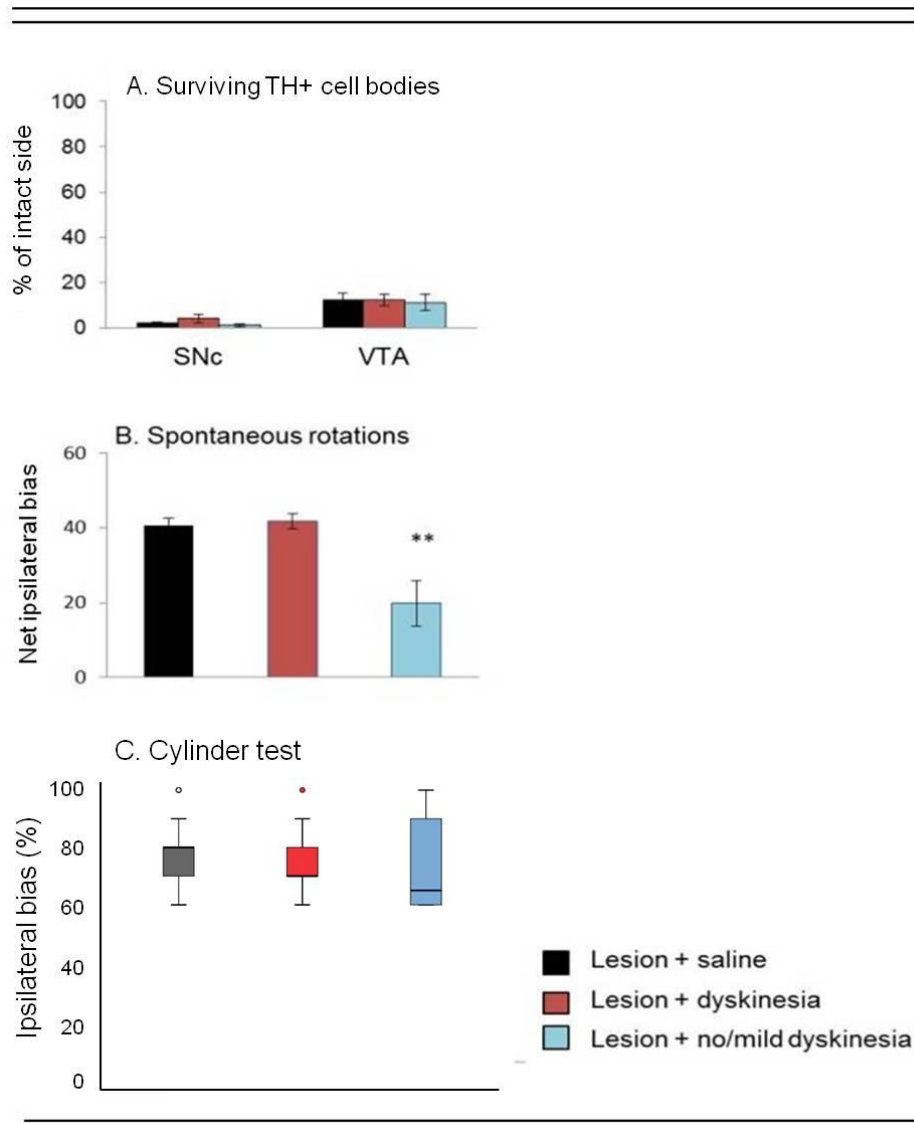


Figure 8.6. Surviving dopaminergic cell bodies in the lesion SNc and VTA, expressed as a percentage of the intact side (A), together with the net ipsilateral bias of rats' spontaneous rotations (B), and the ipsilateral bias on the cylinder test (C) of lesion rats treated with saline (n=42) as well as lesion, L-DOPA treated rats classified as dyskinetic or non-dyskinetic (n=42 and 9). The bar charts (A,B) show the mean, and the error bars the standard error of the mean. The boxplot (C) shows the median and interquartile ranges. SNc=Substantia nigra pars compacta; TH=Tyrosine hydroxylase, VTA=Ventral tegmental area. * $p < 0.05$, ** $p < 0.01$

LCRT task movement time. An initial analysis including both ipsilateral and contralateral performance was conducted. The intact groups required a similar amount of time when

responding to cues presented on the ipsilateral and contralateral side to the lesion. Conversely, all lesion groups took significantly longer to respond to contralateral cues (Figure 8.7A-B; Side x Group: $F_{3,144}=8.52$, $p<0.001$; Simple Effect: $\min.F_{1,144}=10.64$, $p<0.01$). While intact rats' movement times were shorter than those of lesion rats, there was no overall difference between lesion rats that did or did not develop LID following L-DOPA treatment (Group $F_{3,144}=56.16$, $p<0.001$).

Because the lesion deficit manifested itself on the contralateral side, a further analysis restricted to contralateral performance was conducted. This analysis demonstrated that lesion dyskinetic rats were significantly slower than the other lesion groups, which did not differ significantly from each other (Group: $F_{3,145}=41.48$, $p<0.001$). There was a gradual increase in the time taken to execute contralateral responses in all lesion rats, apart from the L-DOPA treated non-dyskinetic cohort (Figure 8.7B; Day: $F_{4,580}=4.83$, $p<0.01$; Day x Group: $F_{12,580}=5.15$, $p<0.001$; Simple Effects: $\min.F_{4,142}=9.27$, $p<0.001$).

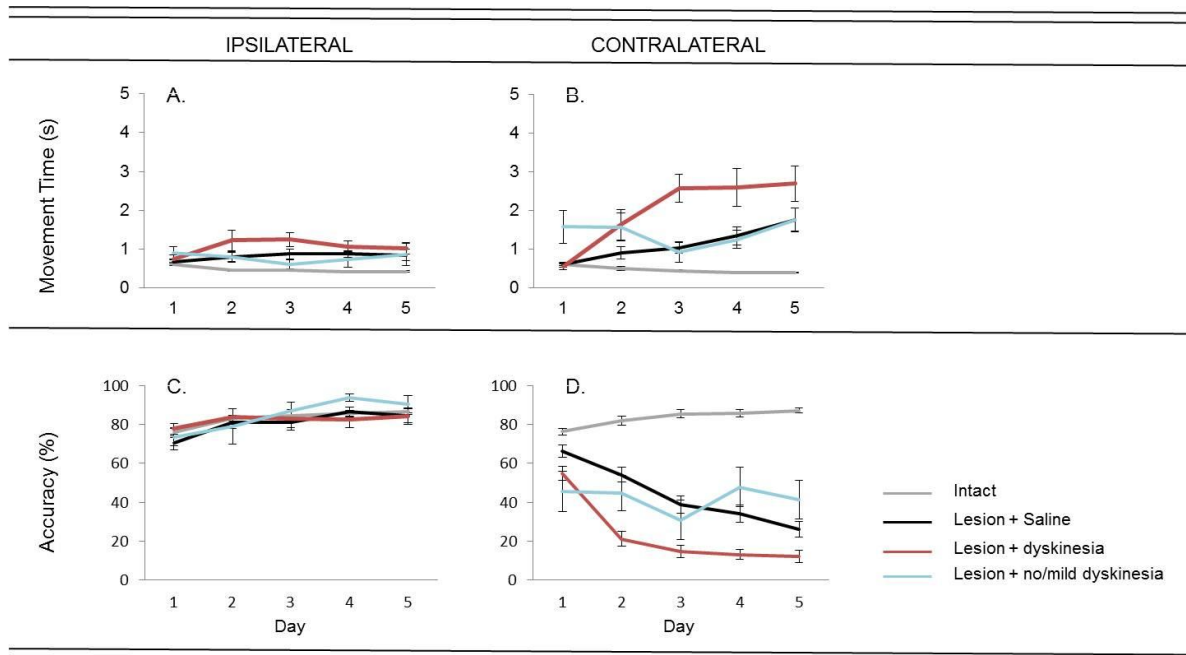


Figure 8.7. The ipsilateral and contralateral movement time (A-B) and accuracy (C-D) of intact (n=56), lesion saline treated (n=42), lesion, L-DOPA treated dyskinetic (n=42), and lesion, L-DOPA treated non-dyskinetic rats (n=9) tested on the LCRT task. The line chart shows the mean response on each testing day, and the error bars show the standard error of the mean. LCRT=Lateralised choice reaction time task.

LCRT task accuracy. Intact rats' accuracy levels were similar when responding to ipsilateral and contralateral cues. Conversely, lesion rats were less accurate when responding to cues

presented contralateral than ipsilateral to the lesion (Figure 8.7C-D; Group x Side: $F_{3,144}=103.47$, $p<0.001$; Side x Group x Day: $F_{12,576}=10.37$, $p<0.001$; Simple Effect of Side: $\min.F_{1,144}=9.63$, $p<0.01$). Chronic L-DOPA treatment further impaired the lesion induced deficit in rats that developed LID in response to L-DOPA. Notably, the dyskinetic cohort showed a greater decrease in their contralateral accuracy than lesion L-DOPA treated rats that remained non-dyskinetic (Group: $F_{3,144}=104.20$, $p<0.001$).

8.3.4.2. Comparison of rats with different levels of AIMS

To test whether AIMS magnitude, rather than LID onset, impaired LCRT task performance, lesion L-DOPA treated rats were divided into four groups depending on what quartile their AIMS scores fell into. This created four lesion L-DOPA treated groups; 13 rats with AIMS $<1^{\text{st}}$ quartile (median: 1, range: 0-2.5); 13 rats with AIMS between the 1^{st} and 2^{nd} quartile (median: 8.5, range: 3-52.5), 13 rats with AIMS between the 2^{nd} and 3^{rd} quartile (median: 85.5, range: 58-105), and 12 rats with AIMS $>3^{\text{rd}}$ quartile (median: 131.5, range 111 to 154.5). It should be noted that 9 out of the 13 rats in the AIMS $<1^{\text{st}}$ quartile group were classified as non-dyskinetic in the analysis described in the previous section (8.3.4.1).

Lesion effects. As previously stated, the lesion induced a significant loss of dopaminergic cell bodies in the ipsilateral SNc (Figure 8.8A; $t_{92}=-29.74$, $p<0.001$) and VTA ($t_{92}=-33.83$, $p<0.001$). An further analysis comparing the percentage dopamine loss observed in the groups' ipsilateral SNc and VTA confirmed that the lesion groups did not differ in the extent of their dopamine loss (Group: $F_{4,88}=0.54$, n.s.) which, for both groups, was more pronounced in the SNc than in the VTA (Region: $F_{1,88}=33.07$; Region x Group: $F_{4,88}=0.52$, n.s.). All groups showed a strong ipsilateral bias on the spontaneous rotation and cylinder tests. Lesion L-DOPA rats whose AIMS scores were $>3^{\text{rd}}$ quartile showed a significantly greater bias in their spontaneous rotations than lesion L-DOPA treated rats whose AIMS were below $<1^{\text{st}}$ quartile. None of the other groups' spontaneous rotations differed from each other (Figure 8.8B; Group: $F_{4,88}=3.06$, $p<0.05$). There was no difference in the ipsilateral bias the lesion groups exhibited on the cylinder test (Figure 8.8C; $H=8.26$, $df=4$, n.s.).

LCRT task movement time. An initial analysis including both ipsilateral and contralateral performance was conducted. Intact rats took were faster than lesion rats at executing responses to visual cues. However, there was no overall difference between the movement time of the four lesion groups (Figure 8.9A; Group: $F_{5,143}=12.13$, $p<0.001$). Because movement times were significantly longer when responding to cues presented on the

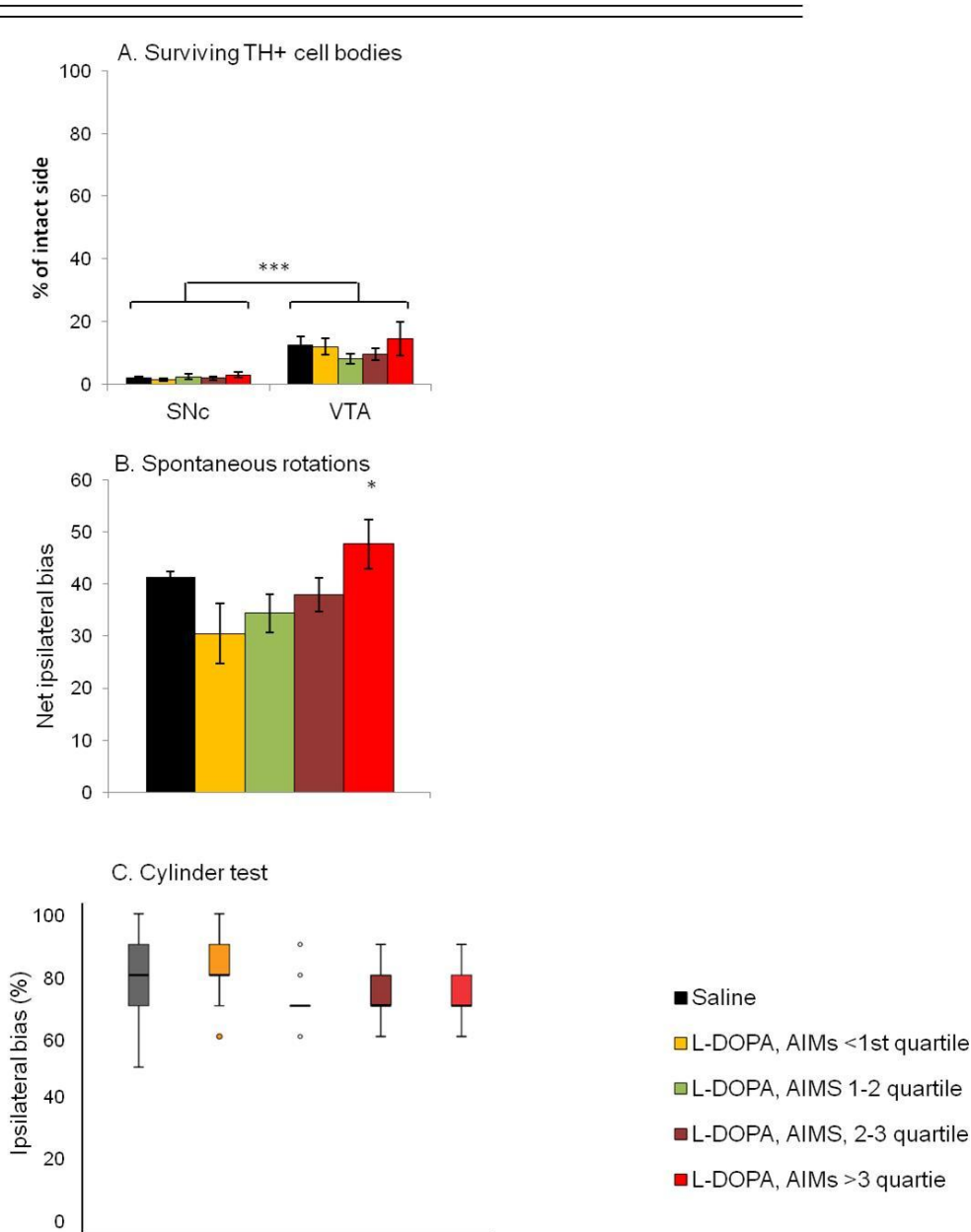


Figure 8.8. Surviving dopaminergic cell bodies in the lesion SNc and VTA, expressed as a percentage of the intact side (A), together with the net ipsilateral bias of spontaneous rotations (B), and ipsilateral bias on the cylinder test (C) of lesion rats treated with saline (n=42) as well as lesion L-DOPA treated rats whose AIMs were below the 1st quartile (n=13), between the 1st and 2nd quartile (n=13), between the 2nd and 3rd quartile (n=13), and above the 3rd quartile mark (n=12). The bar charts (A, B) show the mean and the error bars the standard error of the mean. The box plot (C) shows the median and interquartile ranges with circles representing outliers. AIMs=Abnormal involuntary movements, SNc=Substantia nigra pars compacta, TH=Tyrosine hydroxylase, VTA=Ventral tegmental area. *p<0.05 (versus AIMs <1st quartile group), **p<0.01

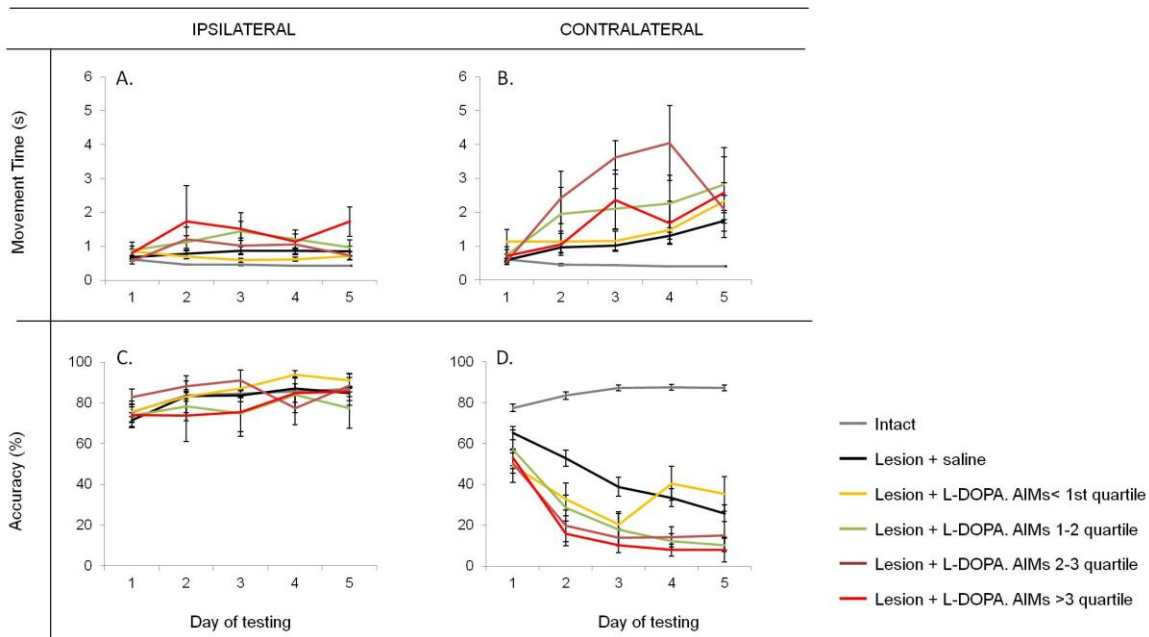


Figure 8.9. The ipsilateral and contralateral movement time (A-B) and accuracy (C-D) of intact (n=56), 6-OHDA + saline treated (n=42) and lesion L-DOPA treated rats whose AIMs were <1st quartile (n=13), between the 1st and 2nd quartile (n=13), between the 2nd and 3rd quartile (n=13), and above the 3rd quartile (n=12). The line charts show the mean response at each testing days, and the error bars show the standard error of the mean. AIMs=Abnormal involuntary movements.

contralateral side (Side: $F_{1,143}=17.40$, $p<0.001$), a second analysis restricted to contralateral movement time was conducted.

While intact rats movement times was stable over the five days of testing there was a gradual increase in the contralateral movement time of all lesion groups (Figure 8.9B; Day x Group: $F_{20,572}=2.55$, $p<0.001$; Simple Effect: min. $F_{4,140}=2.59$, $p<0.01$). The intact rats were, overall, faster at responding to contralateral cues than lesion rats, but there was no difference between the four lesion, L-DOPA treated groups (Group: $F_{5,143}=9.71$, $p<0.001$).

LCRT task accuracy. An initial analysis including both ipsilateral and contralateral accuracy was conducted. In this analysis, all lesion groups were less accurate than intact controls but did not differ from each other (Figure 8.9C-D; Group: $F_{5,143}=40.49$, $p<0.001$). The lesion induced deficit specifically manifested itself on when rats were required to respond to contralateral cues (Side x Group: $F_{5,143}=42.54$, $p<0.001$; Simple Effect: min. $F_{1,143}=74.02$, $p<0.001$).

A second analysis, that was restricted to the contralateral side, was therefore conducted. When responding to cues presented on the contralateral side, the lesion groups'

performance decreased over the five-testing days whereas the intact rats' accuracy remained stable (Figure 8.9D; Day x Group: $F_{20,572}=8.68$, $p<0.001$; Simple Effect: $\min.F_{4,140}=5.32$, $p<0.05$). The lesion saline treated group had greater contralateral accuracy than all lesion L-DOPA treated groups apart from the groups whose AIMs fell below the first quartile and predominantly consisted of non-dyskinetic rats (Group: $F_{5,143}=123.81$, $p<0.001$).

8.3.4. Discussion

The aim of the analysis was to determine whether there was a dissociation between the LCRT task performance of lesion L-DOPA rats that did, or did not, develop LID. The data confirmed that these groups did differ significantly from each other. When comparing dyskinetic and non-dyskinetic rats, a further decrease in contralateral accuracy only occurred in lesion rats that developed LID following L-DOPA treatment. Importantly, when comparing lesion L-DOPA treated rat with different levels of AIMs magnitude, only the performance of lesion L-DOPA treated group whose AIMs were $<1^{\text{st}}$ quartile, and predominantly consisted of non-dyskinetic rats, aligned with the lesion saline treated rats' LCRT task performance. All other L-DOPA treated groups showed an exacerbation of the lesion induced contralateral accuracy deficit. This suggests that the L-DOPA deficit only manifested itself in rats that developed LID, and that once rats had developed LID the magnitude of their AIMs did not further impact on LCRT task performance.

All lesion groups showed an equal dopamine denervation, and the dissociation between dyskinetic and non-dyskinetic rats' LCRT task performance could therefore not be explained in terms of lesion extent. It is, however, worthwhile to mention that higher AIMs scores were associated with a greater ipsilateral bias in rats' spontaneous rotations (Figure 8.8). This is in line with previous findings of a significant correlation between spontaneous rotations and AIMs (discussed in Experiment 2) but is unlikely to have impacted on the accuracy of LCRT task responding as previous publications have demonstrated that the accuracy deficit in the LCRT task is not driven by a motor impairment (Dowd & Dunnett, 2005a,b).

8.4. Chapter discussion & conclusions

The purpose of this chapter was to test whether the previously observed effect of chronic L-DOPA on LCRT task performance (Chapter 7) was due to chronic L-DOPA exposure or the onset of LID. The pooling of data from two LCRT task experiments demonstrated that an exacerbation of the lesion induced accuracy decline only occurred in lesion L-DOPA rats that

developed LID, whereas their non-dyskinetic counterparts did not differ from saline treated controls. Thus, the data suggest that the impairment was linked to molecular or cellular changes associated with LID onset.

The LCRT task accuracy deficit has previously been suggested to reflect extinction (Dowd & Dunnett, 2004, 2007). Based on data showing that a role of hippocampal LTP induction in fear extinction (Farinelli *et al*, 2006) it was hypothesised that the restored ability for cortico-striatal LTP that is observed following chronic L-DOPA treatment to lesion rats enhanced extinction learning - and that this was the cause of the exacerbation of the contralateral accuracy deficit observed in Chapter 7. In addition to restored cortico-striatal LTP, chronic L-DOPA also abolishes the ability for depotentiation in rats that develop LID in response to the L-DOPA treatment, but not in rats that remain non-dyskinetic (Picconi *et al*, 2003). The induction of depotentiation has previously been demonstrated to disrupt extinction learning (Farinelli *et al*, 2006). One may thus hypothesise that the non-dyskinetic rats, in which the ability for cortico-striatal depotentiation is retained (Picconi *et al*, 2003), would show less marked extinction than their dyskinetic counterparts. The current data supported this hypothesis by showing a greater decrease in the contralateral accuracy of the dyskinetic than the non-dyskinetic lesion cohort. However, it is acknowledged that, while the findings presented in this chapter support the hypothesis, it was beyond the scope of the presented experiments to fully determine the nature of the LCRT task deficit or directly test the mechanism underlying the phenomenon. Thus, while the depotentiation/extinction hypothesis may explain the difference in dyskinetic and non-dyskinetic rats' LCRT task accuracy it requires further testing.

To test the validity of the hypothesis, and to increase understanding of the nature of the observed LCRT task deficit, future experiments should directly test effect of LID onset on lesion rats' extinction rates. This could, for example, be conducted in an extinction study in which four groups of rats (intact; lesion, drug naive; lesion, L-DOPA treated and dyskinetic; lesion, L-DOPA treated and non-dyskinetic) first undergo an acquisition period during which they learn to that perform nose pokes in an operant chamber when a light cue is present in exchange for the delivery of a sucrose pellet. Once the CS-US association has been acquired, a subset of the each group should undergo an extinction period during which the light cue is no longer predictive of a sucrose reward. Thereafter, the success of the extinction learning should be tested during a retention trial, during which all rats are re-tested on the task in the same environment as they first learned the CS-US association. Successful extinction learning should cause the subgroup of rats that have undergone extinction training to make fewer lever

presses during the retention trial than control rats. Furthermore, if LID onset increases extinction learning as previously hypothesised, it is predicted that lesion dyskinetic rats that have undergone extinction testing (i) show a faster rate of extinction, and (ii) make fewer responses during the retention trial than lesion L-DOPA treated, non-dyskinetic rats that have also undergone extinction testing. Assuming such an effect was to occur, further tests should be conducted to determine whether the behaviour meet the criteria for extinction. This includes demonstrating (i) that extinguished rats show *spontaneous recovery*, i.e. exhibit the CR when retested in the same environment after a test-free period, (ii) that extinguished rats show *renewal*, i.e. exhibit the CR if the retention test takes place in a different environment than the extinction test (e.g. by changing the appearance of the operant chamber by putting striped wallpaper on its walls), and (iii) that extinguished rats show *reinstatement*, i.e. exhibit the CR during a retention trial if they are exposed to the US between the extinction and retention trials.

The main finding of the data presented in this chapter was that LID onset is associated with the development of non-motor symptoms in the rat 6-OHDA model of PD. Current literature predominantly considers LID to be a motor complication that manifest itself when patients are on dopaminergic medication (e.g. Jenner, 2008). However, preclinical and clinical data demonstrating that LID onset is also associated with sensitisation of striatal dopamine receptors (Konradi *et al*, 2004; Aubert *et al*, 2005) and disrupted synaptic plasticity (e.g. Picconi *et al*, 2003, Huang *et al*, 2011) suggest that LID onset may also affect non-motor behaviour mediated by the striatal circuitry, and that these effects may be independent of the acute effects of L-DOPA. The data presented in this chapter and the previous chapter builds on these earlier studies by demonstrating that LID onset does affect non-motor behaviour in a rat PD model. Hence, the data challenge the view of LID as a pure motor complication and suggest that long-term L-DOPA treatment affects a wider range of behaviours than previously recognised. Furthermore, the data show that unlike dyskinetic movements, the effect of LID onset on non-motor behaviour is present even when lesion rats are off medication. While acute L-DOPA is already known to both improve motor function (Birkmayer & Hornykiewicz, 1961) and impacting on non-motor function such as visual attention (Schneider *et al*, 2013; Keri *et al*, 2013) and learning (e.g. Cools *et al*, 2006), the current data shows that chronic L-DOPA also affect both motor and non-motor functions. The clinical implications of these findings and suggestions for future research to be conducted in this field are further discussed in Chapter 9.

9. General discussion

L-DOPA is the gold standard treatment for PD but long-term use is associated with the development of adverse motor side effects. While *acute* L-DOPA is known to affect both motor and non-motor function, little is known about the effects of *long-term* L-DOPA on non-motor behaviour. The aim of this thesis was to explore the effects of long-term L-DOPA treatment on motor and non-motor function to determine whether, like acute L-DOPA, chronic treatment has dissociable effects on motor and non-motor behaviour.

To inform the design of subsequent experiments, the experiments presented in Chapters 3 to 4 aimed to (i) identify non-pharmacological lesion screening tests that would not risk sensitising the dopaminergic system, and thereby potentially confounding later data on the interaction between L-DOPA and behaviour, and (ii) determine acute L-DOPA doses that could be administered to lesion, food restricted rats with a history of chronic L-DOPA treatment prior to testing in operant chambers without the presence of interfering dyskinesias. Together, these experiments provided data to guide methodological decisions in later experiments.

A subsequent series of experiments tested the hypotheses that chronic L-DOPA affects (i) the acquisition of novel motor skills using the staircase task (Chapter 6), and (ii) non-motor functions measured in the LCRT task (Chapters 7 and 8). After identifying an effect of chronic L-DOPA on LCRT task performance, the effects of chronic versus acute administration of L-DOPA and a D2 agonist were compared. Having established that the effect on non-motor function was only observed in lesion rats treated with L-DOPA, the effect of LID onset on non-motor function was explored using an anti-dyskinetic compound and by pooling data from earlier experiments. Together, these data demonstrated (i) that chronic treatment with L-DOPA but not with a D2 agonist affects non-motor function, and (ii) that the observed effects of chronic L-DOPA on non-motor function only occurs in lesion rats that develop LID as a result of the treatment.

9.1. Nutritional issues in PD

Diet is known to affect the pharmacokinetics of L-DOPA, with dietary neutral amino acids competing with L-DOPA for transport across both the wall of the small intestine and the BBB (reviewed in del Amo *et al*, 2008). Some patients suffering from L-DOPA induced motor complications are therefore recommended special diets to reduce these motor complications. While early stage PD patients have been encouraged to continue consuming

normal protein intake (15% of the total energy), a 5% reduction in protein consumption has been recommended for late stage PD patients suffering from L-DOPA induced motor complications. In addition, late stage patients have been recommended to redistribute their protein intake to consume less protein during breakfast and lunch and more protein during dinner, as well as taking their medication approximately 30 min prior to meals (Barichella *et al*, 2009; Cereda *et al*, 2010).

Based on literature showing that LAT mediated transport of L-DOPA is affected by the levels of dietary amino acids competing for transport (Kageyama *et al*, 2000), it was therefore hypothesised that lesion food restricted rats would show a greater motor response to acute L-DOPA than *ad libitum* controls (Chapter 4). The data showed increased motor response to acute L-DOPA in food restricted rats with intra-striatal, but not MFB, lesions. To determine the mechanism underlying this phenomenon, a microdialysis study was conducted (Experiment 5). Contrary to what had been hypothesised, the microdialysis data did not demonstrate increased influx of L-DOPA in food restricted rats. However, the data did suggest that food restriction affected the serotonergic system. In particular, there were increased 5HIAA levels in the lesion striata of 6-OHDA MFB lesion rats relative to their *ad libitum* controls. This suggested an increased serotonergic activity following food restriction in a rat model of late stage PD. The data were in line with earlier reports of an increased serotonin turnover in intact male rats following food restriction (Schweiger *et al*, 1989) and therefore expanded on current knowledge by demonstrating a similar effect in the ipsilateral striata of rats with near complete dopamine loss.

Interestingly, a systematic review has reported that while protein redistribution diets improve PD patients' motor function and decrease off-time, some patients on these diets require a decrease in their L-DOPA dose due to a worsening of their dyskinesias (Cereda *et al*, 2010). Preclinical data have linked dyskinesias to the uptake, and subsequent uncontrolled release, of L-DOPA by serotonergic neurons (Carta *et al*, 2006; Carlsson *et al*, 2007). A role of serotonin in LID has also been demonstrated by the anti-dyskinetic effects of 5HT1A/B antagonists (Bédard *et al*, 2013), and the ability of 5HT1 agonists to reduce the magnitude of AIMs as well as the development of LID in rodent and primate models of PD (Lundblad *et al*, 2005; Eskow *et al*, 2007; Muñoz *et al*, 2008) and to reduce AIMs in patients (Bonifati *et al*, 1994). Against this background, it could be queried whether food restriction could indirectly affect AIMs magnitude via increases in serotonergic activity. Future studies may wish to explore this by replicating the finding of increased 5HIAA levels presented in Chapter 5, while extending on the data by also measuring serotonin levels and doing

systematic AIMS scoring of food restricted and *ad libitum* rats following acute L-DOPA injection.

In addition to AIMS, serotonin affects a range of other motor and non-motor behaviours. For example, co-administering a 5HT2 agonist with a D1 agonist enhances dopamine agonist-induced locomotion in 6-OHDA lesion rats (Bishop & Walker, 2003). This effect is likely linked to the stimulation of 5HT2C receptors, as intra-striatal antagonism of 5HT2C receptors decreases baseline levels of striatal dopamine (Lucas *et al*, 2000). Serotonin also affects non-motor behaviour, as demonstrated by e.g. data showing impaired inhibition of incorrect responding on a visual discrimination following striatal and extrastriatal serotonin loss (Harrison *et al*, 1999). Hence, while food restriction is often required in operant paradigms, the data in Chapter 5 suggest it may impact on performance in tasks that are reliant on the serotonergic system and highlights the importance of consistent food restriction protocols in such experiments.

9.2. Effects of acute dopaminergic treatment on motor and non-motor function

Acute L-DOPA is known to affect motor and non-motor behaviour differently. While it improves motor symptoms of PD (Birkmayer & Hornykiewicz, 1998; Miyasaki *et al*, 2002), it also interferes with a number of non-motor functions in patients and animal models of PD.

For example, Decamp & Schneider (2009) demonstrated that acute L-DOPA impairs MPTP lesion rhesus monkey's performance on a delayed response task which was designed to measure executive and attentional function. These findings have since been replicated in MPTP lesion macaques (Schneider *et al*, 2013) and have been hypothesised to be caused by overdosing of the frontal cortex, which is less affected by MPTP lesions than the striatum (Decamp & Schneider, 2009). Similar effects have been observed in patients. Cools and colleagues (2010) compared the performance of PD patients tested on or off dopaminergic medication on a delayed response task in which patients were shown visual stimuli that they were instructed to recall, immediately followed by visual distractors that were either congruent or incongruent with the stimuli. For the majority (87%) of patients, their medication consisted of L-DOPA while the remaining patients received dopamine agonists. Patients tested off medication were less affected by the presentation of congruent distractors than healthy controls, but their performance aligned with that of controls when tested on medication (Cools *et al*, 2007). Hence, dopaminergic medication increased the interfering effect of congruent distractors, thereby making patients' performance similar to that of

healthy participants when tested on medication. The same delayed response task has previously been associated with neural activity in the striatum and prefrontal cortex (Cools *et al*, 2007), and it was hypothesised that the effect of PD on performance was due to an imbalance between striatal and frontal cortical dopamine levels, which resulted in abnormal resistance to distractors (Cools *et al*, 2010). An interesting study, related to Cools and colleagues' (2010) hypothesis, was conducted by Keri and colleagues (2013) who described the effects of a dopamine agonist and L-DOPA treatment on PD patients' ability to recall pictures associated, or not associated, with a target letter. Prior to testing, participants were told they would be shown a series of photos with different backgrounds and instructed to remember a white target letter, that would be superimposed on one of these photos, while ignoring and forgetting a black distractor letter that would be superimposed on another photo. Immediately after a target letter recall test, patients were shown two photos, of which only one had been presented to them during the task, and asked which of the photos they had seen before. When on medication, patients were better than healthy controls at identifying photos that had been shown at the same time as either the target or distractor letter. Crucially, there was no difference between participants' ability to identify photos that had been shown in the absence of a target or distractor letter, suggesting that the phenomenon was not due to enhanced memory *per se* (Keri *et al*, 2013). Instead, Keri and colleagues (2013) hypothesised that the dopaminergic medication provided an 'attentional boost' that helped direct attention to items associated with a cue. Together, these studies suggest that acute dopaminergic medication interferes with PD patients' performance on delayed response tasks by affecting the stimuli which patients attend to (Cools *et al*, 2010; Keri *et al*, 2013). This may sometimes impair patients' performance by focusing their attention on irrelevant stimuli (e.g. Cools *et al*, 2010), and has been hypothesised to be caused by overdosing of the prefrontal cortex (Decamp & Schneider, 2009; Cools *et al*, 2007)

In addition, acute dopaminergic medication also affects learning. Acute L-DOPA has been shown to increase learning from positive feedback while decreasing learning from negative feedback (e.g. Cools *et al*, 2006; Frank *et al*, 2004). It has also been demonstrated that patients tested on medication (a combination of L-DOPA and dopamine agonists) perform worse than unmedicated patients in terms of the reversal, but not the acquisition, of a discrimination rule (Swainson, *et al*, 2000). The impairing effect of dopaminergic medication (L-DOPA and agonists, with or without a MAO inhibitor) on reversal learning has also been demonstrated by other groups (Cools *et al*, 2001). Performance on reversal learning tasks has been shown to rely on the prefrontal cortex, ventral striatum, and orbito-frontal cortex

(Kehagia *et al*, 2010). Because motor symptoms of PD are primarily due to dopamine loss in the dorsal striatum, it has been hypothesised that the L-DOPA doses required to replace the lost dopamine in the dorsal striatum simultaneously over-stimulate the ventral striatum (Cools, 2006). This, in turn, has been hypothesised to increase reward-like signals and decrease error-like signals in the ventral striatum, which is believed to hinder learning from negative feedback and thereby impair reversal learning (Cools, 2006). Similar to the interfering effect of L-DOPA on visual attention, the effect of the drug on learning is thus believed to stem from the fact that the doses required to restore dopamine levels in the dorsal striatum overdose other, less denervated, structures.

9.3. Effects of chronic dopaminergic treatment on motor and non-motor function

It has long been known that chronic L-DOPA treatment induces motor side effects in the form of dyskinesias and “off” periods (Marsden & Parkes, 1977). However, less is known about the effects of long-term dopaminergic treatment on non-motor function.

One group of side effects that have received a lot of attention are impulse control disorders (ICDs) which are defined in the American Psychiatric Association’s *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV) as being characterised by an inability, or severe difficulty, in resisting impulses that will result in negative consequences. The prevalence of diagnosed ICDs differs across countries, between approximately one tenth and one quarter of PD patients, with the largest study to date (DOMINION study, n=3090) reporting a prevalence of 13.6% in North American PD patients. ICDs may occur following L-DOPA therapy but are predominantly linked to dopamine agonist use (Weintraub *et al*, 2010; Perez-Lloret *et al*, 2012; Poletti *et al*, 2013). As many other non-motor effects of dopaminergic medication, the development of ICDs has been hypothesised to be due to overdosing of the ventral striatum (Voon *et al*, 2011). In addition, personality traits such as novelty seeking and impulsive behaviour are known to increase the risk for ICDs (Voon *et al*, 2010) which suggest that ICDs in PD may arise from an interaction between a genetic or biological predisposition, and exposure to dopaminergic medication. Furthermore, the type of ICD expressed by patients (e.g. hypersexuality versus compulsive shopping) appears to differ between men and women (Weintraub *et al*, 2010) suggesting that cultural or biological factors also affect the expression of ICDs.

While several longitudinal studies on PD patients have been conducted, these have revealed little on the long-term effects of L-DOPA on non-motor function. For example,

Kulisevsky and colleagues (2000) tested a group of newly diagnosed PD patients with a battery of neuropsychological tests both prior to their first dopaminergic medication and 24 months later. Half of the patients received L-DOPA as a monotherapy throughout the study while the remaining half received a dopamine agonist during the first 6 months, after which L-DOPA was added to their daily medication and co-administered throughout the remainder of the study. Relative to baseline, patients treated with L-DOPA showed decreased performance on the Wechsler Adult Intelligence Scale and a verbal learning task at the end of the study (Kulisevsky *et al*, 2000). However, because there was no treatment naive control group, it is difficult to infer whether the decrease in performance was due to the dopaminergic treatment or disease progression. Furthermore, while one patient developed dyskinesia and another patient was reported to show ‘mild dyskinesia’ at the end of the study the sample size did not allow a comparison between dyskinetic and non-dyskinetic patients, and the data was not segmented according to presence of dyskinesia. Considering that approximately half of PD patients develop LID after 5 years of L-DOPA treatment (Nutt, 2005), a longer follow-up time and segmentation of the data according to presence of dyskinesia would be needed to determine whether LID onset affected neuropsychological performance in Kulivesky and colleagues’ (2000) study.

A similar problem is encountered when attempting to infer the effect of chronic L-DOPA from other published studies. For example, Relja and colleagues (2006) compared neuropsychological test performance of 25 PD patients treated with L-DOPA at two time points, 6 months apart. However, while patients had been diagnosed with PD approximately 5 years prior to the first test session, suggesting that a subsample of the patients would have developed LID during or prior to the study, the data was not stratified according to the presence of dyskinesias. Hence, while an effect of chronic L-DOPA on non-motor function may be present in patients, no clinical studies specifically designed to test this hypothesis have to my knowledge been reported. Therefore, based on currently published data it cannot be determined whether or not the findings of a LID effect on non-motor behaviour presented in Chapters 7 and 8 are representative of a phenomenon also present in PD patients.

While clinical data is ultimately essential for understanding the relevance of the data presented in this thesis to PD patients, there is a theoretical ground for hypothesising that the findings will translate to the clinic. The expression of AIMs in the 6-OHDA MFB rat model used in the thesis project correlates with molecular markers associated with LID in patients such as FosB, prodynorphin, and preproenkephalin (Andersson *et al*, 1999, Winkler *et al*, 2002). The model has also been shown sensitive to the clinically available anti-dyskinetic

drug Amantadine (Lundblad *et al*, 2002; Breger *et al*, 2013). Hence, while the 6-OHDA lesion model does not capture the full PD pathology, it is phenotypically and behaviourally similar to the dyskinesia observed in PD patients.

It is recognised that LID may also be induced in other species, such as mice (e.g. Lundblad *et al*, 2005) and primates (e.g. Bédard *et al*, 1986), and that these models were deliberately not used in this project. It could be argued that a mouse model would be more clinically valid as e.g. mice over-expressing alpha synuclein (Masliah *et al*, 2000) or transgenic LRRK2 mice (Li *et al*, 2009) bear similarities to the human condition that are not captured in the 6-OHDA rat model. However, the rat model was preferred because (i) the primary aim with the presented experiments was to test the hypothesis that LID induction affects non-motor function, rather than studying the interaction between chronic L-DOPA and genetic predispositions for PD, and (ii) the rat model has been more extensively studied, for example in terms of the altered synaptic plasticity associated with PD and LID (Picconi *et al*, 2002). In terms of primate models, it is recognised that these, due to their closer evolutionary relationship with humans, are better preclinical models than the 6-OHDA rat. However, due to the ethical implications and the high cost involved in primate research, these studies are restricted in terms of the number of animals that may be utilised in individual experiments. If a primate model of PD and LID had been used, it would thus not have been possible to conduct the large scale experiments that were required to include the necessary experimental and control groups in this project.

While it is hypothesised that the findings presented here will translate to patients it is also recognised that this requires much further testing. Ultimately, to test the hypothesis it will be necessary to conduct clinical studies that compare neuropsychological performance of dyskinetic and non-dyskinetic PD patients who are matched on other disease parameters, socio-demographic parameters, and preferably also their medication. When comparing such cohorts it will be crucial to utilise an appropriate neuropsychological test. One task of interest could be the Wisconsin Card Sorting Task in which participants are required to match test cards to reference cards depending on their characteristics (e.g. colour, shape, or number of stimuli present on the cards). Participants receive feedback after each trial, indicating whether they matched the test card correctly. Once a participant has learned the rule governing the matching of test and reference cards, the rule is changed without prior notification and the participant is required to use the feedback provided after each trial to learn the new matching rule. In healthy participants, data from fMRI experiments have demonstrated that both the prefrontal cortex and the basal ganglia are activated when participants receive feedback

indicating that a new rule should be learned and during subsequent trials in which participants apply the new rule (Monchi *et al*, 2001). With preclinical studies showing impaired depotentiation in the cortico-striatal circuitry after LID onset (Picconi *et al*, 2003) as well as a role of depotentiation in extinction (Farinelli *et al*, 2003) which reflects relearning of a previously learned association (Myers & Davis, 2002), it is possible that the Wisconsin Card Sorting Task could be sensitive to the non-motor effects of LID induction and therefore useful in clinical studies designed to test the effect of LID onset on patients' non-motor function.

9.4. LID: more than just a motor side effect?

The findings presented in this thesis suggest that the effects of chronic L-DOPA extend beyond merely inducing motor side effects to also affect non-motor function. The phenomenon was present both following overnight withdrawal (Experiment 10), which is the withdrawal period used to study “off” behaviour in clinical studies (e.g. Cools *et al*, 2001; Kulisevsky *et al*, 1996), and following several weeks of L-DOPA withdrawal (Chapter 7). Hence, unlike dyskinesias, the effect was observed even after L-DOPA treatment ceased, suggesting that the reported phenomenon may be of a chronic nature. Should the phenomenon be replicated in clinical studies it will raise the question of whether or not patients will prefer L-DOPA to other anti-Parkinsonian treatments that are less effective in treating motor symptoms, but that hold a lower risk of inducing non-motor side effects. Furthermore, it will suggest a greater need for anti-dyskinetic drugs that do not only reduce the expression of dyskinesias but that prevent the molecular and cellular changes associated with the onset of LID. The only clinically available anti-dyskinetic drug today is the NMDA antagonist Amantadine. While successfully reducing the severity of AIMs exhibited by patients (e.g. Sawada *et al*, 2010) it is not indicated for the prevention of LID onset. If, as hypothesised, it is the pathological changes associated with the *onset* of LID that underlies the non-motor deficit demonstrated in Chapters 7 and 8, Amantadine is thus unlikely to alleviate the non-motor effects of chronic L-DOPA treatment.

9.5. Conclusions

This thesis demonstrated a dissociation between the acute and chronic effects of dopaminergic medication in the rat 6-OHDA model of PD. The main finding was a decreased LCRT task performance in lesion rats that developed LID following L-DOPA treatment, but not in rats that remained non-dyskinetic. This is to my knowledge the first data to show an

effect of LID onset on non-motor function. As such, the data suggest that the neurobiological changes associated with LID onset affect a wider range of behaviour than previously recognised, and therefore extends our understanding of the side effects induced by L-DOPA treatment. It is hoped that future experiments will further investigate this phenomenon: both by identifying the exact neuropsychological mechanisms underlying the LID-induced deficit, and by determining whether non-motor effects of LID onset are also observed in patients.

10. References

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Appendix I

The following section provides an overview of the statistical analysis supporting the decision to pool the intact groups in section 7.4. The data were analysed using a repeated measures ANOVA with Day as within subject factor and Group as a between subject factor. Significant between-subject differences were analysed using a Scheffe post-hoc test. The analysis included six experimental groups (intact /lesion with saline/L-DOPA/bromocriptine).

Usable trials: There was an increase in the number of usable trials made by intact rats over the five days of testing, while the number of usable trials made by lesion rats decreased over the same period of testing (Day: $F_{4,316}=117.06$, $p<0.01$; Day x Group: $F_{20,316}=2.84$, $p<0.001$; Simple Effect: $\min.F_{4,76}=5.66$, $p<0.001$). However, there was no difference between the number of usable trials made by intact rats treated with saline, L-DOPA, or bromocriptine (Group: $F_{5,79}=17.46$, $p<0.001$).

Reaction time: While there were some fluctuations in the recorded reaction time over the course of testing this was independent of lesion and drug treatments, as well as of what side the CS was presented on (Day: $F_{4,316}=3.39$, $p<0.05$; Day x Group: $F_{20,316}=1.31$, n.s.; Side x Day: $F_{4,316}=1.71$, n.s.; Side x Day x Group: $F_{20,316}=1.16$, n.s.; Side: $F_{1,79}=2.47$, n.s.; Side x Group: $F_{5,79}=0.75$, n.s.). There was no difference in the ipsilateral reaction time exhibited by the six groups (Group: $F_{5,79}=0.93$, n.s.)

Movement time: Lesion rats were slowed than intact controls, but there was no difference in the performance of the three intact groups (Group: $F_{5,79}=12.29$, $p<0.01$). Lesion rats treated with bromocriptine showed longer movement times when responding to contralateral than ipsilateral cues on all days of testing apart from day 4, while lesion rats treated with L-DOPA and saline showed an ipsilateral bias on all, apart from the second and fourth, day of testing. Intact rats treated with bromocriptine took marginally longer to respond to ipsilateral than contralateral cues on the first day of testing but did not show a bias on any of the remaining testing days. No ipsilateral bias was observed in intact rats treated with saline or L-DOPA (Side x Day x Group: $F_{20,316}=2.21$, $p<0.01$; Simple Effect: $\min.F_{1,79}=4.20$, $p<0.05$).

Accuracy: While all intact groups were more accurate when responding to contralateral than ipsilateral cues on the first day of testing, they did not exhibit a side bias on any of the other testing days. Conversely, lesion rats treated with saline or L-DOPA were

consistently less accurate when responding to contralateral than ipsilateral cues. A similar ipsilateral bias in responding was observed in lesion rats treated with bromocriptine on all apart from the first day of testing (Side x Day x Group: $F_{20,316}=7.55$, $p<0.001$; Simple Effect: $\min.F_{1,79}=5.10$, $p<0.05$). Overall there was a difference between the accuracy of intact and lesion groups, but not difference in the accuracy with which the three intact groups responded to cues ($F_{5,79}=16.19$, $p<0.001$).

Appendix II

The following section provides an overview of the statistical analysis supporting the decision to pool the intact groups in section 8.2. The data was analysed using a repeated measures ANOVA with Day as a within-subject factor and Lesion, TAT/SDV and L-DOPA/saline as between-subject factors.

Usable trials: There was no difference in the usable trials made by rats treated with the TAT2A peptide or the inactive SDV vehicle (TAT: $F_{1,81}=0.14$, n.s.; TAT x Lesion: $F_{1,81}=1.23$, n.s.; TAT x L-DOPA: $F_{1,81}=0.18$, n.s.; TAT x Lesion x L-DOPA: $F_{1,81}=0.25$, n.s.; TAT x Day: $F_{4,324}=0.90$, n.s.)

Ipsilateral reaction time: The reaction time observed following presentation of cues ipsilaterally to the lesion was not similar for all groups (TAT: $F_{1,81}=0.50$, n.s.; TAT x Lesion: $F_{1,81}=2.06$, n.s.; TAT x L-DOPA: $F_{1,81}=1.34$, n.s.; TAT x Lesion x L-DOPA: $F_{1,81}=0.88$, n.s.; TAT x Day: $F_{4,324}=0.21$, n.s.)

Contralateral reaction time: TAT treatment did not impact on the reaction time observed following presentation of contralateral cues (TAT: $F_{1,81}=0.69$, n.s.; TAT x Lesion: $F_{1,81}=1.39$, n.s.; TAT x L-DOPA: $F_{1,81}=1.05$, n.s.; TAT x Lesion x L-DOPA: $F_{1,81}=1.32$, n.s.; TAT x Day: $F_{4,324}=1.78$, n.s.).

Ipsilateral movement time: TAT treatment did not impact on the time taken for rats to execute responses to ipsilateral cues (TAT: $F_{1,81}=1.00$, n.s.; TAT x Lesion: $F_{1,81}=0.27$, n.s.; TAT x L-DOPA: $F_{1,81}=0.73$, n.s.; TAT x Lesion x L-DOPA: $F_{1,81}=0.76$, n.s.; TAT x Day: $F_{4,324}=0.68$, n.s.)

Contralateral movement time: TAT treatment did not impact on the time taken for rats to execute responses to contralateral cues (TAT: $F_{1,81}=0.32$, n.s.; TAT x Lesion: $F_{1,81}=0.00$, n.s.; TAT x L-DOPA: $F_{1,81}=0.08$, n.s.; TAT x Lesion x L-DOPA: $F_{1,81}=0.06$, n.s.; TAT x Day: $F_{4,324}=0.75$, n.s.)

Ipsilateral accuracy: The accuracy with which rats responded to cues presented ipsilateral to the lesion was not affected by TAT2A treatment (TAT: $F_{1,81}=0.78$, n.s.; TAT x Lesion: $F_{1,81}= 3.39$, n.s.; TAT x L-DOPA: $F_{1,81}=0.77$, n.s.; TAT x Lesion x L-DOPA: $F_{1,81}=0.21$, n.s.; TAT x Day: $F_{4,324}=0.50$, n.s.)

Contralateral accuracy: The accuracy with which rats responded to cues presented contralateral to the lesion was not affected by TAT2A treatment (TAT: $F_{1,81}=0.06$, n.s.; TAT x Lesion: $F_{1,81}=0.57$, n.s.; TAT x L-DOPA: $F_{1,81}=0.51$, n.s.; TAT x Lesion x L-DOPA: $F_{1,81}=0.15$, n.s.; TAT x Day: $F_{4,324}=1.19$, n.s.).