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Sensorimotor Cueing Mechanisms and Neocortical Neuroplasticity in a Rat Model of Parkinson's Disease

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Sensorimotor Cueing Mechanisms and Neocortical Neuroplasticity in a Rat Model of Parkinson's Disease" submitted by Andrew Brown in partial fulfilment of the requirements of the degree of Master of Science.

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Abstract

Parkinson's disease involves nigrostriatal degeneration leading to impaired movement. Sensory cues can facilitate movement in Parkinson's disease, but little is known on the mechanisms involved. A behavioural model in the rat was developed to assess whether prior auditory cue training is associated with changes in neocortical field responses and reduced akinesia from haloperidol challenge. Auditory tone, but not thalamic stimulation, cue training significantly increased the evoked thalamo-auditory response and increased performance in the learned task under haloperidol challenge that was not due to a general reduction in akinesia. In a second study, the assumption of frontal activity dysfunction from dopamine depletion was investigated by assessing forelimb neocortical movement representation expression in the rat with intrastriatal 6-OHDA infusion and under acute haloperidol. Dopamine antagonism from bilateral, but not unilateral, lesions or haloperidol significantly reduced forelimb map area. Results suggest cortical function is altered following cue training and from dopamine antagonism.

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List of Symbols, Abbreviations and Nomenclature

Symbol	Definition
6-OHDA	6-hydroxydopamine
A8	Retrorubral dopamine cell group projection
A9	Nigrostriatal dopamine cell group projection
A10	Mesolimbic dopamine cell group projection
ANOVA	Analysis of variance
AP	Anterioposterior
CFA	Caudal forelimb area
CM	Centromedian nucleus of the thalamus
D1	Dopamine type-1 receptor
D2	Dopamine type-2 receptor
DAB	3,3'diaminobenzidine tetrahydrochloride
DBS	Deep brain stimulation
DV	Dorsoventral
EEG	Electroencephalogram
EtOH	Ethanol
GPe	Globus pallidus external segment
GPi	Globus pallidus internel segment
i.p.	Intraperitoneal
ICMS	Intracortical microstimulation
L-DOPA	3,4-dihydroxy-L-phenylalanine
MGv	Medial geniculate ventral division
ML	Mediolateral
MPP+	1-methyl-4-phenylpridinium
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MSN	Medium spiny neuron
NaOH	Sodium hydroxide
NiCl	Nickel chloride
PD	Parkinson's disease; Paralysis agitans
PPN	Pedunculopontine nucleus
PSB	Phosphate buffered solution
RFA	Rostral forelimb area
SEM	Standard error of the mean
SNc	Substantia nigra pars compacta
SNr	Substantia nigra pars reticulata
SPL	Standard pressure level
STN	Subthalamic nucleus
TE1	Primary auditory field/cortex
TH	Tyrosine hydroxylase
VA	Ventral anterior nucleus of the thalamus
VL	Ventrolateral nucleus of the thalamus
VTA	Ventral tegmental area

Chapter 1: Introduction

Parkinson Disease (PD, *paralysis agitans*) is a late-onset and progressive neurodegenerative disorder. PD has a mean age of onset of 57 years of age (Koller et al., 1987) and affects approximately 1-2 % of the general population over 65 years, increasing to 5% at 85 years (Fahn, 2003). It is the second most common neurodegenerative disorder following Alzheimer's (Bower et al., 1999), and these numbers are likely to increase substantially in the coming decades as the population ages (Kincannon et al., 2005). The vast majority of PD cases (> 90%) are sporadic, with no known genetic linkage; although several monogenetic mutations associated with rare familial forms of the disorder have recently been identified (Wider and Wszolek, 2007; Lesage and Brice, 2009). Cardinal clinical characteristics of the disorder include slow resting tremor, muscle rigidity, akinesia, bradykinesia, and gait disturbances with postural instability (Lang and Lozano, 1998; Olanow et al., 2001). Nonmotor manifestations present as autonomic and olfactory dysfunction, depression, dementia, and sleep disturbances (see Simuni and Kapli, 2008 for a comprehensive review). Pathological hallmarks are characterized by a profound and preferential degeneration of melanized dopaminergic neurons of the substantia nigra pars compacta (SNc) projecting to the striatum coupled with intraneuronal proteinaceous inclusions (Lewy Bodies; Forno, 1996; Braak, et al., 2003).

1.0 Pathology

PD is primarily characterized by the progressive loss of dopamine neurons in the SNc forming the nigrostriatal dopamine pathway (Figure 1-1), and the presence of fibrillar, proteinaceous cytoplasmic inclusions in remaining cells termed Lewy bodies when present in parikarya and Lewy neurites when present in neuronal processes (Lewy, 1912; Gibb, 1991; Forno, 1996; Figure 1-2). There are three main mesencephalic dopaminergic cell populations: the A8 group of the retrorubral and lateral reticular areas projecting to the ventrocaudal putamen, the A9 group in the SNc projecting to the dorsal putamen, and the A10 group in the ventral tegmental area projection to the nucleus accumbens (Dahlstöm and Fuxe, 1964; McRitchie et al., 1996). Nigrostriatal (A9) dopamine neurons are preferentially lost in PD, with extranigral dopamine groups showing much less degeneration (Uhl et al., 1985; Hirsch et al., 1988; Forno, 1996). Due to topographical projections of the various dopamine cell groups and greater insult to the nigrostriatal pathway, dopamine depletion in PD is most pronounced in the putamen (Berheimer et al., 1973). Symptoms do not manifest until approximately 50% of SNc neurons have died and striatal dopamine levels fall by 80% (Hornykiewicz, 1986). The large reserve capacity of the nigrostriatal system is presumed to be mediated by compensatory responses to reduced dopaminergic tone from both increased activity of remaining SNc efferents and increased postsynaptic striatal dopamine receptor density and sensitivity (Deumens et al., 2001).

Although nigrostriatal degeneration is commonly considered to be the most pronounced marker of the disease, extensive extranigral pathology is also observed. Cell loss and Lewy inclusions are known to occur throughout the brain in PD including the dorsal motor nucleus of the vagus, reticular formation, raphe, locus coeruleus, nucleus

Figure 1-1: Representation of the nigrostriatal pathway (left) originating from substantia nigra pars compacta (SNc) dopamine neurons projecting to the putamen and caudate nucleus of the striatum (solid lines). Line thickness represents the degree of nigrostriatal innervation. Marked degeneration of SNc neurons to the putamen (dashed line) and to a lesser extent the caudate nucleus (thin line) is observed in Parkinson's disease (right). Note loss of neuromelanin-pigmented nigrostriatal SNc neurons in Parkinson's disease in photograph (black arrows). Adapted from Dauer and Przedborski, 2003.

Figure 1-1: Nigrostriatal degeneration in Parkinson's disease

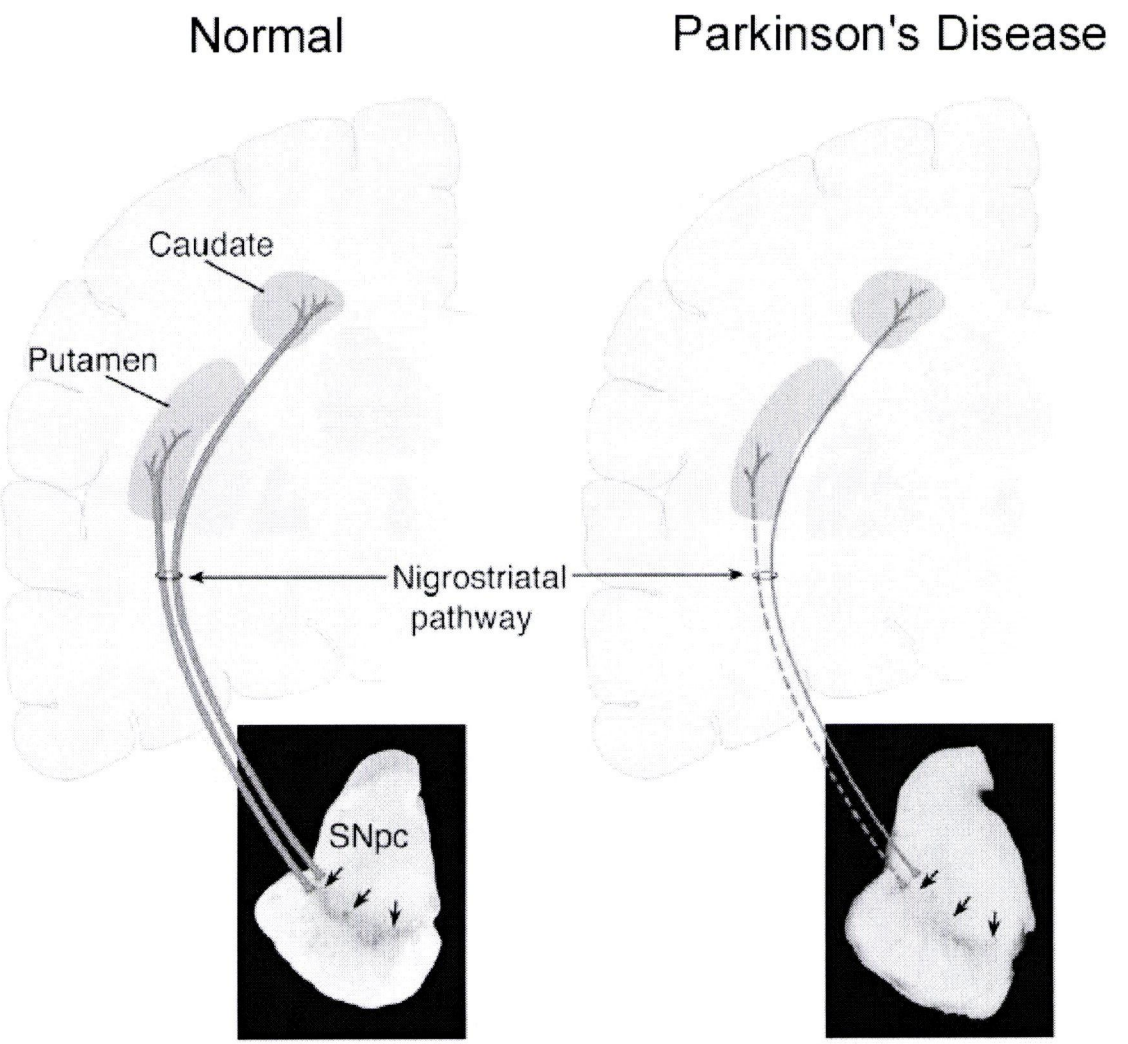
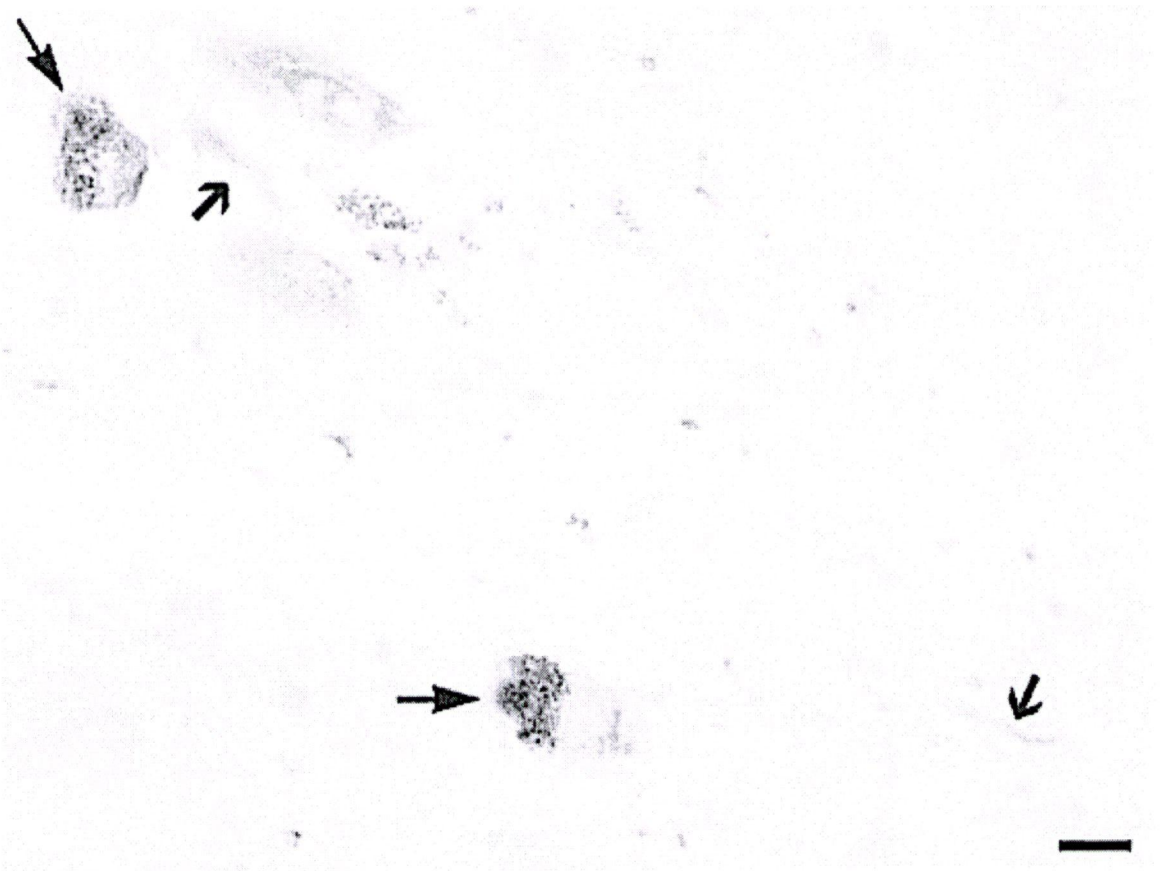


Figure 1-2: α-Syncluein immunostain of substantia nigra pars compacta from Parkinson's disease patients (from the MRC Cambridge Brain Bank). Lewy bodies in two neurons (thin arrows), and Lewy neurites in neuronal processes (thick arrows) are observed. Scale bar: 20 μm. Adapted from Spillantini et al., 1997.

Figure 1-2: Lewy bodies and neurites in the substantia nigra in Parkinson's disease



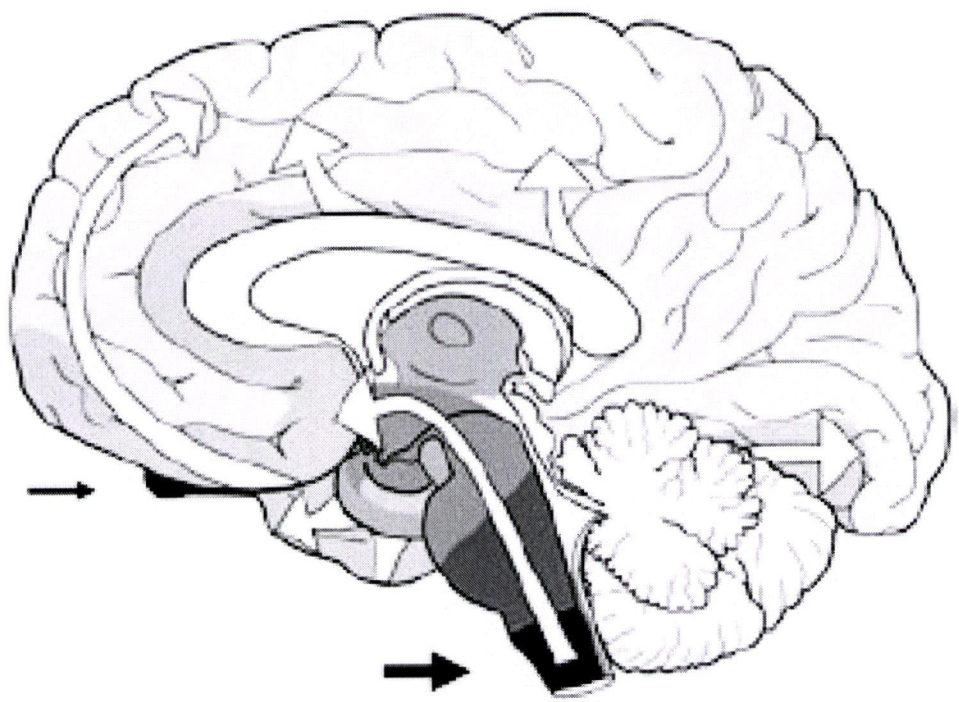
basalis of Meynert, olfactory bulb, autonomic nervous system, and widespread cortical areas (Hornykiewicz and Kish, 1987; de Vos et al., 1995; Braak and Braak, 2000; Braak et al., 2003). Moreover, Braak et al (2003) have proposed a pathological staging of PD emphasizing a caudo-rostral progression from the brainstem to neocortex, with SNc involvement occurring only in the midcourse of the disease (Figure 1-3). While extranigral pathological contributions to disease symptomology are not fully understood, they appear to primarily involve nonmotor manifestations (Simuni and Sethi, 2008; Ahlskog, 2008).

1.1 Pathogenesis

Although rare forms of PD (< 10% of cases) have been associated with single gene defects; the cause of the sporadic condition is unknown and it is unclear as to how much of the disease results solely from either environmental factors, genetic causation, or, more realistically, a combination of the two (Steece-Collier et al., 2002). Causal theories suggest a genetic susceptibility to exogenous or endogenous toxic agents that lead to oxidative damage and mitochondrial dysfunction in cell populations that may be predisposed to oxidative stress. Although the initial insult that causes neurodegeneration in PD may differ between sporadic and inherited forms, there is indication that downstream pathogenic processes may be common to both. Converging evidence has implicated oxidative stress and mitochondrial dysfunction as well as protein misfolding and aggregation in the pathogenesis of the disorder (Dauer and Przedborski, 2003; Gandhi and Wood, 2005; Hawkes et al., 2007).

Figure 1-3: Schematic representation of Lewy body progression in Parkinson's disease. Inclusions are first observed in the dorsal motor nucleus of the vagus in the medullary hindbrain (thick black arrow). Pathology then progresses in a caudo-rostral direction (dark to light regions indicating progression from hindbrain to forebrain structures). The olfactory bulb (thin black arrow) is also an initial site of inclusion pathology. Adapted from Braak et al., 2003.

Figure 1-3: Lewy body inclusion progression in Parkinson's Disease



1.1.1 Environmental toxins

The finding that acute intoxication with MPTP can cause motor deficits indistinguishable from PD (Langston, 1985) provided initial support for a causative role of environmental factors. The active metabolite of MPTP, MPP+, was originally developed for use as a herbicide (Terzioglu and Galter, 2008) and shares striking similarities to the herbicide paraquat, to which exposure is known to significantly increase the risk of developing PD (Costello et al., 2009). There have been numerous reports of an association between toxin exposure and PD development (Tanner, 1992; Dick, 2006; Ascherio et al., 2006; Dhillon et al., 2008; Costello et al., 2009). While no single, specific agent as been conclusively identified, pesticide exposure is known to significantly increase susceptibility. Although age is the strongest risk factor (Bower et al., 2000; Van Den Eede et al., 2003), the processes causing cell death in PD may be distinct from those of normal ageing. The pattern of nigral cell loss in the disease is concentrated in ventrolateral and caudal regions of the SNc, differing from the dorsomedial degeneration exhibited with normal ageing (Fearnley and Lees, 1991). There is also a marked male predominance, though it is unknown whether this relates to hormonal, environmental, or genetic factors (Bower et al., 2000; Van Den Eede et al., 2003). Further supporting an environmental influence in PD susceptibility are findings of that cigarette smoking and alcohol and coffee consumption are inverse risk factors (Hernan et al., 2002; Hernan et al., 2003).

1.1.2 Endogenous toxin hypothesis

A possible endogenous toxin source to dopamine cell populations is dopamine itself. The dopamine oxidative stress hypothesis proposes that dopamine both enzymatically and spontaneously generates toxic free radicals that play a central causal role in neuronal death in PD (Olanow, 1990, Fahn and Cohen, 1992). This hypothesis is supported by findings of increased oxyradical products in degenerating SNc neurons and in Lewy bodies (Dexter et al., 1989; Giasson et al., 2000). Furthermore, the highest peroxidase activity in the brain is observed in the SNc and is significantly decreased in this area and in the striatum in PD (Ambani et al., 1975). Neuromelanin is abundant in SNc neurons and is the product of dopamine metabolism and oxidation (Zecca et al., 2002). However, there is substantial evidence against the dopamine oxidative stress hypothesis. First, there is an inverse relationship observed in PD between neuromelanization and cell death: dopamine neurons with the most neuromelanin are spared and neurons with the least are preferentially lost. Furthermore, dopamine agonist therapies are not associated with increased toxicity in either animals or PD patients (Murer et al., 1998; Datla et al., 2001; Lyras et al., 2002.). Moreover, a prediction this hypothesis would be that dopaminergic neurons would be the first cell population to be affected and it is known that dopaminergic cell loss starts only in midcourse of disease progression (Braak et al., 2003).

1.1.3 Lewy bodies and PD pathogenesis

Lewy bodies are spherical eosinophilic intraneuronal inclusions displaying an organized structure of a dense hyaline core and diffuse halo surround (Forno et al., 1996). While a definite diagnosis of PD requires the histological presentation of both dopaminergic SNc cell loss and Lewy Body inclusions, the disorder is usually diagnosed on clinical grounds. Although PD is associated with Lewy bodies, it is currently unknown whether they play a causal or consequent in disease aetiology and progression (Dauer and Przedborski, 2003; Takahashi and Wakabayashi, 2005). There is convergent evidence that the degenerative process in PD may involve related features of mitochondrial dysfunction, oxidative stress, and protein misfolding (Dauer and Przedborski, 2003; Gandhi and Wood, 2005; Hawkes et al., 2007). Lewy bodies are known to contain markers of protein misfolding and dysfunctions of the ubiquitin-proteasome system by staining positive for ubiquitin (Kuzuhara et al., 1998). Furthermore, a major component of Lewy bodies is a-synuclein (Spillantini et al., 1998) and a link between a-synuclein aggregation and PD is known in rare familial form of the disorder (Kruger et al., 1998; Polymeropoulos et al., 1997; Singleton et al., 2003; Chartier-Harlin et al, 2004). Additionally, transcriptional upregulation of a-synuclein has been observed in idiopathic PD (Gründemann, et al., 2008). Cytosolic segregation and aggregation of these components into inclusions, however, could simply reflect and adaptive cellular response to limit the effects of ongoing pathological processes. Supporting this interpretation is the finding that the majority of SNc neurons undergoing apoptotic-like cell death in PD do not contain Lewy bodies (Tompkins and Hill., 1997). Furthermore, Lewy body inclusions are conspicuously absent in familial PD cases associated with parkin mutations

(van de Warrenburg et al., 2001), and are not specific to the disorder (Gibb and Lees, 1988; McKeith, 2006).

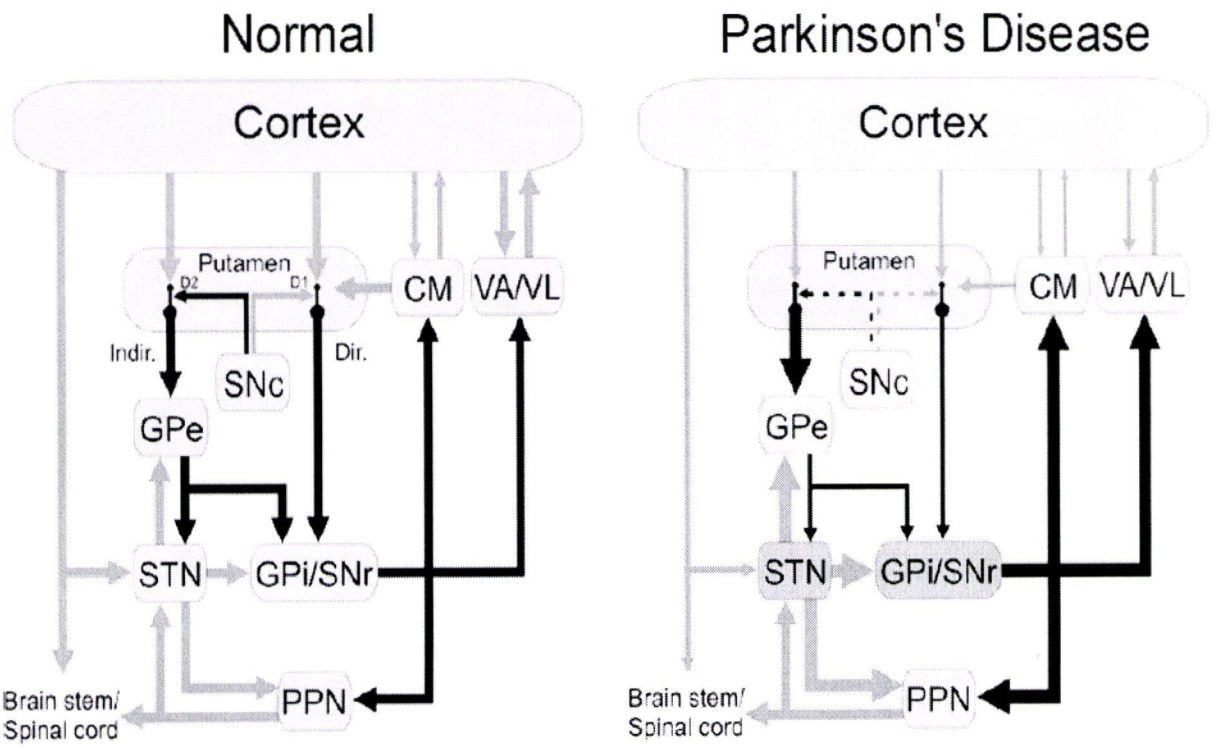
1.2 Pathophysiology

1.2.1 Suppression of thalamocortical activity

The basal ganglia-thalamocortical circuit model of PD proposes that nigrostriatal degeneration leads to increased inhibitory basal ganglia output activity that suppresses thalamocortical projections and results, clinically, in hypokinesia (Albin et al., 1989; Parent and Hazrati 1995; Parent and Cicchetti, 1998; DeLong and Wichmann, 2007; Wichmann and Delong 1998; Wichmann & DeLong 2003; Figure 1-4). The basal ganglia consist of several interconnected nuclei that serve, in part, as an intermediary for cortical-subcortical re-entrant circuits linking the thalamus and neocortex. They receive wide-ranging cortical input and send their output, via the thalamus, to the neocortex and brainstem. There are five separate cortical-subcortical circuits that maintain parallel functional and anatomical segregation throughout their basal ganglia projections: (1) The skeletomotor motor circuit arising from frontal motor regions and involved in voluntary movement; (2) the occulomotor circuit from the frontal eye fields and mediating control of saccadic eye movements and orienting responses; (3) a limbic circuit from the cingulated gyrus and medial orbitofrontal cortex involved in motivational behaviour; and two prefrontal circuits focused on the (4) orbitofrontal and (5) dorsolateral prefrontal

Figure 1-4: Schematic representation of overall activity changes in the basal ganglia-thalamocortical circuit model of Parkinson's disease. Grey arrows depicts excitatory connections and black arrows depict inhibitory connections. Arrow thickness corresponds to the activity magnitude. Nigrostriatal degeneration (dashed lines) in Parkinson's disease is presumed to increase inhibitory basal ganglia output activity (GPi/SNr) that suppress thalamocortical connections (VA/VL) to frontal motor regions, leading to hypokenesia. Abbreviations: CM, centromedian nucleus of the thalamus; Dir., direct pathway; D1, dopamine type-1 receptor; D2, dopamine type-2 receptor; GPe, globus pallidus external segment; GPi, globus pallidus internal segment; Indr., indirect pathway; PPN, pedunculopontine nucleus; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; STN, subthalamic nucleus; VA, ventral anterior nucleus of the thalamus; VL, ventrolateral nucleus of the thalamus. Adapted from Galvan and Wichmann, 2008.

Figure 1-4: Basal ganglia-thalamocortical model of Parkinson's disease



areas, involved in executive and social behaviour functions, respectively (Alexander et al., 1986; Alexander and Crutcher, 1990).

The skeletomotor circuit originates in frontal premotor, supplementary motor, and primary motor regions and projects onto medium spiny GABAergic neurons (MSN) of the striatum forming the input to the basal ganglia (Takada et al., 1998). Striatal MSNs project via two pathways to the internal pallidum (GPi) and the substantia nigra pars reticulata (SNr) which together comprise the basal ganglia output nuclei (GPi/SNr). A 'direct' pathway projects monosynaptically from the striatum to the GPi/SNr. An 'indirect' pathway passes to the GPi/SNr via the external pallidum (GPe) and the subthalamic nucleus (STN). The STN-GPi/SNr projection is glutamatergic and forms the only intrinsic excitatory pathway of the basal ganglia; all others are inhibitory and GABAergic. The circuit is then closed by inhibitory GPi/SNr efferent projections to ventrolateral thalamic areas that impinge back onto frontal neocortical motor regions. The GPi/STN tonically inhibit their thalamic efferents (Delong and Whichmann, 1993). Due to the number and polarity of the connections in these two pathways, stimulation of the direct pathway facilitates thalamocortical activity and movement by disinhibition of the GPi/SNr; whereas stimulation of the indirect pathway suppresses thalamocortical transmission and inhibits movement by disinhibition of the STN and increased inhibitory outflow from the GPi/SNr.

In addition to glutamatergic projections from the neocortex, striatal activity is further modulated by dopamine projections from the SNc. Most striatal MSNs express distinct postsynaptic dopamine receptors: those in the direct pathway express D1 receptors and those in the indirect pathway express D2 receptors. Dopamine exerts a

differential effect on these two receptor subtypes, exciting MSNs expressing D1 receptors and inhibiting MSNs expressing D2 receptors (Gerfen et al., 1990). The effect of dopamine input to the striatum is increased transmission in the motor circuit via facilitation of conduction in the direct pathway and inhibition of conduction in the indirect pathway. According to the basal ganglia-thalamocortical circuit model, loss of dopaminergic input to the striatum then results in decreased activation of the 'direct' pathway which leads to increased inhibitory GPi outflow onto thalamocortical connections. In contrast, loss of dopaminergic input to the striatum results in decreased inhibition of the 'indirect' pathway which disinhibits the STN and leads to increased activation of inhibitory GPi outflow onto thalamocortical connections. Together, loss of striatal dopamine in both pathways results in overactivity of basal ganglia output, reduced activation of the ventrolateral thalamus, and suppression of activity in frontal motor cortical areas. Hyperactivity in the STN and GPi in the Parkinsonian state is paramount to this circuit model and is supported by electrophysiological (Bergman et al., 1994), metabolic (Eidelberg et al., 1994), and imaging studies (Crossman, 1985).

Although the basal ganglia-thalamocortical circuit model provides a simplified account for motor dysfunction in PD that serves as the basis for current pharmacological and surgical therapies (Olanow, 2004; Benabid, 2009), several limitations have become apparent (Obeso et al., 1997; Braak and Del Tredici, 2008). The first comes from conflicting findings of GPe activity in the Parkinsonian state. The model stipulates that striatal dopaminergic depletion would result in GPe hypoactivity; however, increases (Bezrd et al., 1999), decreases (Filion and Tremblay, 1991) and no changes (Levy et al., 1997) in GPe activity have been reported. GPe hypoactivity in the model is thought to

lead to STN overactivity, and overactivity of the STN has been perhaps the most consistent finding in PD pathophysiological research (Obeso et al., 2000). However, Hassani et al (1997) have observed increased STN activity following nigrostriatal dopamine lesions that is independent of disinhibition from the GPe. Additionally, Obeso et al. (2008) note projections from the cortex, thalamus, and pedunculopontine nucleus (PPN) that could be responsible for STN activity changes following striatal dopamine depletions. STN hyperactivity may then be accounted for in the model despite equivocal reports of changes in GPe activity.

1.2.2 Oscillatory activity

A further limitation of the basal ganglia-thalamocortical model comes from its inability to account for the fact that lesions of the thalamus do not lead to significant bradykinesia (Fox et al, 1991; Brophy et al., 1997) and that pallidotomy is not associated with development of dyskinesias due to overactivity of thalamocortical projections (Lang et al., 1997; Baron et al., 2000). To account for such findings, Marsden and Obeso (1994) propose that perhaps it is the alteration in discharge pattern, rather than discharge rate emphasized in the basal-ganglia thalamocortical circuit model, that may exert a greater influence on movement impairment in PD. Supporting this interpretation are findings of enhanced beta band (10-35 Hz) oscillatory activity in the basal ganglia and frontal motor cortical areas associated with hypokinesia and rigidity phenomena in PD (Brown and Marsden, 1998; Gatev et al., 2006). Dopamine loss is also associated with increases in synchronous activity in the STN, GP, and SNR (Filion and Tremblay, 1991; Wichmann

and Soares, 1996; Heimer et al., 2006). Importantly, dopamine therapy or therapeutic deep brain stimulation (DBS) of the STN appears to suppress both motor dysfunction and pathological neural oscillations in a resetting of basal ganglia activity to higher frequency (60-90 Hz) uncorrelated firing patterns (Brown et al., 2001; Bergman et al., 1994). Furthermore, increased beta band coherence between neocortical EEG activity and local field potentials in the STN is observed in PD and there is an increased correlation between neocortical EGG, and single cell activity in the STN and pallidum (Wichmann et al., 2002). Thus, increased synchrony of basal ganglia and neocortical neural activity at lower frequencies is associated with a hypokinetic effect. It is currently unknown exactly how dopamine depletion induces synchronization in basal ganglia nuclei, but increased electronic coupling between adjacent striatal cells (Onn and Grace, 1999) or axon collateral activity may be involved (Guzmen et al., 2003).

1.3 Symptomatic treatments

Although PD was originally characterized by James Parkinson in 1817, an effective symptomatic treatment was not available until the 1960's when converging investigations lead to the discovery that dopamine replacement therapy can greatly improve motor dysfunction. First, Carlsson et al., (1959), mapping out ascending catecholamine projection systems, noticed that the vast majority of dopamine (~80 %) is localized in the basal ganglia and that experimental degeneration of the nigrostriatal system in animals resulted in PD-like symptoms (Anden et al., 1964; Carlsson et al, 1964). Degeneration of the nigrostriatal pathway was soon observed in PD patients and

dopamine replacement therapy using L-DOPA was shown to significantly improve hypokinesia (Hornykiewicz, 2002). Reestablishing equilibrium in basal ganglia circuitry either by pharmacological (Lewitt, 2008) or surgical (Lozano & Synder, 2008; Reza et al., 2008) intervention has been the primary therapeutic approach in Parkinson's disease treatment and has significantly improved the quality of life for many patients. However, no current treatment either slows or reverses disease progression.

1.3.1 Dopamine agonism

L-DOPA (3,4-dihyroxy-L-phenylalanine) therapy has revolutionized PD treatment (Lewitt, 2008), significantly reducing morbidity and mortality (Sweet and Mc Dowell, 1975; Joseph et al., 1978). L-DOPA, a dopamine metabolite, is administered orally and is actively transported into circulation, crossing the blood brain barrier and being converted into dopamine by aromatic l-amino acid decarboxylase in residual nigrostriatal fibres (Hornykiewicz, 2002). L-DOPA therapy results in significant improvements in akinesia, bradykineia, and muscle rigidity; although resting tremor sometimes remains (Yahr and Duvoisin, 1972). To avoid nausea and vomiting side effects from activation of dopamine receptors in the area postrema, unprotected by the blood-brain barrier, carbidopa is commonly co-administered to reduce peripheral metabolism of L-DOPA (Olanow, 2004).

A limitation of L-DOPA therapy is that while it provides symptomatic motor relief, the underlying neurodegenerative progression is not hindered. Additionally, chronic treatment is associated with the development of motor complications. While L-

DOPA has a fairly short half-life (60-90 minutes), it provides long acting facilatory motor effects in the initial stages of treatment with no interdose loss of effectiveness, even when administered two times daily (Schapira et al., 2008). During initial stages of the disease, long-duration responses to L-DOPA without clinical fluctuations have been observed days (Hauser et al., 1994) and even weeks (Fahn et al., 2004) following cessation of treatment. During continued L-DOPA use, however, motor fluctuations develop in which symptomatic effectiveness is lost a few hours following administration ("wearing off") and motor symptoms re-emerge prior to the next dosing (Nutt and Holford, 1996). With continued chronic administration, the acute duration of L-DOPA effectiveness progressively decreases to the half-life of the drug. Motor fluctuations due to wearing-off effects are common with chronic L-DOPA therapy; a recent literature review estimated the frequency of motor complications to be 3% following one year of therapy, 41% after six years, and 70% after nine years (Ahlskog and Muenter, 2001). Chronic L-DOPA treatment is also associate with the development of sever dyskinesias. Choreic, dystonic, ballistic, and stereotypic involuntary movements occur mostly during times of peak L-DOPA plasma concentrations (Muenter and Tyce, 1971). As with motor fluctuations, an increase in dyskinesia risk is associated with continued L-DOPA treatment. Dyskinesias from continued L-DOPA use is common and estimated to be present in 26% of patients after 5 years of treatment, and 43% after ten years (Kumar et al., 2005; Van Gerpen et al., 2006).

The mechanisms of L-DOPA induced motor complication development are not fully understood; though fluctuating plasma levels that result in pulsatile, intermittent striatal dopamine receptor stimulation are suggested to be a primary factor (Olanow et al.,

2006). Motor complications can be effectively controlled for the most part by reducing the L-DOPA dosing interval, through use of controlled-release L-DOPA preparations, the use of adjunctive dopaminergic agonist treatments, and by reducing dopamine metabolism with either catechol-O-methyltransferase of monoamine oxidase-B inhibitors (Lewitt, 2008).

1.3.2 Surgical intervention

Pallidotomy and ventral thalamotomy have been long used in the treatment of PD (Wycis et al., 1957; Ohye et al., 1964) While effective at reducing resting tremor and rigidity phenomena, these surgical interventions have little effect on akinesia and bilateral lesions are associated with unacceptable side-effects including hemorrhage and dysarthria. With the advent of L-DOPA therapy surgical ablation fell into relative disfavor but has undergone a popular reassurance in recent times due to refinements in stereotaxic techniques (Laitinen et al., 1992).

A recent development in neurosurgery is the discovery that chronic high-frequency deep brain stimulation (DBS) can functionally and reversibly mimic the effects of target ablation. Initially performed in the thalamus to reduce tremor (Benabid et al., 1993), DBS of either the pallidum (Krack et al., 1998) or STN (Benabid et al., 2009) has proved effective against all PD symptoms. While DBS can provide benefit for motor dysfunctions in PD, its effects are no greater than what can be achieved with L-DOPA alone (Fahn, 2008). Moreover, patients unresponsive to L-DOPA therapy do not benefit greatly from the procedure (Welter et al., 2002). DBS candidates, therefore, are those

who develop sever motor complications rather than L-DOPA unresponsive patients (Ahlskog, 2007).

1.3.3 Sensorimotor cueing

Novel rehabilitative therapies for motor impairment in PD focus on an intriguing phenomenon of the disorder in which patients, often suffering from considerable akinesia, can initiate movements with greater velocity, force, and accuracy in response to an external cue than they would be able to voluntarily (Schwab, 1959; Martin, 1967). A striking example of this 'Paraxodical kinesia' is given by Forssberg et al (1984) who observed that simply placing pieces of white paper in front of patient can result in a doubling of stride length. Similarly, auditory cues have also been shown to improve both gait and movement kinematics (McIntosh, Brown, Rice, & Thaut, 1997; Howe, 2003; Ma, Trombly, Wagenaar, & Tickle-Degnen, 2004).

The freezing and festination of gait in PD a debilitating motor dysfunction that causes a profound impact in quality of life (Martinez-Martin, 1998) and is very difficult to manage therapeutically (Rascol et al., 2000; Giladi et al., 2001). Recent reports have shown that physiotherapy employing sensory cueing techniques can be a powerful means of improving gait and reducing hypokinesia in PD (Rubinstein et al., 2002; Morris et al., 2008) that can be used in conjunction with pharmacological therapy. While the mechanisms underlying polysensory cued movement activation in PD have not been fully elucidated, they have been proposed to be of extra-basal ganglia origin involving

cerebellar-parietal-premotor and non-lemniscal thalamic circuitry (Hu, 2003; Kagerer et al., 2003; Debaere et al., 2003; Samuel et al., 1997).

1.4 In vivo experimental models

Animal models are important and valuable tools in which to study pathogenic mechanisms involved in human disease and to evaluate therapeutic strategies. Current *in vivo* animal models of PD focus on either administration of exogenous toxins that cause SNc cell loss or genetic mutations associated with rare familial forms of the disease. Presented below is a summary of the most commonly used toxin models of idiopathic PD and common models of established familial PD for which transgenic animals have been developed and rigorously assessed.

1.4.1 Toxin models

1.4.1.1 6-OHDA

6-hydroxydopamine (6-OHDA) was the first chemical known to exert a specific neurotoxic effect on catecholamine neurons (Ungerstedt, 1968). 6-OHDA is a hydroxylated analogue of dopamine and is selective taken up by catecholamine transporters (Luthman et al., 1989). It induces cell death via oxidative stress from the generation of hydrogen peroxide and hydroxyl radials (Cohen and Werner, 1994; Kumar et al., 1995). 6-OHDA does not cross the blood-brain barrier and must be stereotaxically infused. Application of 6-OHDA into the lateral ventricles produces widespread central

catecholamine depletion (Ungerstedt, 1968; Bloom et al., 1969) and noradrenergic cell loss can be prevented by pretreatment with a norepinephrine transporter inhibitors. Nigrostriatal degeneration can be induced by local 6-OHDA infusion into SNc (Carman et al., 1991), the striatum (Kirik et al., 1998), or the medial forebrain bundle (Ungerstedt, 1968). The majority of studies utilize unilateral 6-OHDA infusion (Deumens et al., 2202) as it results in asymmetric circling behavior that is correlated with lesion extent and provides quantitative behavioural assessment of the lesion with ease to screen potential therapeutic treatments. When challenged with drugs acting on the dopaminergic system, imbalances in basal ganglia output activity between the intact and lesion hemispheres result in rotation away from the side of greater activity (Ungerstedt, 1971). Bilateral 6-OHDA lesions provide a closer approximation to PD (Van Oosten et al., 199) and although large bilateral lesions are usually associated with greatly increased aphagia, adipsia and mortality (Deumens et al., 2002); partial bilateral (Linder et al., 1999) or double bilateral (Ben et al., 1999) intrastriatal lesions can be performed without decreased animal viability.

1.4.1.2 Rotenone

Pesticides and environmental toxins have been suggested to be involved in PD pathogenesis (Tanner, 1992; Dick, 2006). Rotenone is a naturally occurring compound produced in the roots of certain plant species that is commonly used as a natural insecticide and piscicide (Hisata, 2002). Rotenone exerts toxic effects by inhibiting both complex I of the mitochondrial electron transport chain (Schuler and Casida, 2001) and microtubule formation (Marshall and Himes, 1978). Chronic systemic exposure to

rotenone has been shown in rats to result in progressive degeneration of nigrostriatal neurons and induce α-synuclein aggregation and Lewy body-like formation (Betarbet et al., 2000; Sherer et al., 2002). While initial reports indicated selectivity for nigrostriatal neurons, perhaps suggesting particular sensitivity of this population to mitochondrial dysfunction, resent studies indicate more widespread pathology (Höglinger et al., 2003). Limitations of the model include high mortality and large individual variability in nigrostriatal pathology (Höglinger et al., 2003; Sherer et al., 2003; Lapoint et al., 2004). Furthermore, rotenone has been shown to also induce severe peripheral pathology (Betarbet et al., 2000; Lapointe at al., 2004) including dramatic weight loss and muscle wasting that could influence motor dysfunction. None the less, rotenone is an important model as it is associated with PD-like pathology induced by a naturally occurring chemical and is a valuable tool for investigating the relationship between protein aggregation and cell death.

1.4.1.3 Proteasomal inhibition

Deficits in the ubiquitin-proteasome system have been implicated in the pathogenesis of both familial and sporadic PD (Dawson and Dawson, 2003; McNaught and Olanow, 2003). Proteasomal inhibition in rats may produce what is perhaps the best toxin model of PD to date. Systemic administration of naturally occurring (epoxymicin) or synthetic (PSI) proteasome inhibitors has been shown to induce a progressive model in rats that recapitulates the core behavioural and pathological features of the disorder. Following an initial incubation latency, progressive motor dysfunction (bradykinesia, rigidity, and tremor) and a pattern of cell loss and a-synuclein containing Lewy body-like

inclusions mimicking that in PD is observed in the substantia nigra, locus coeruleus, nucleus basalis of Meynert, and dorsal motor nucleus of the vagus (McNaught et al., 2004). Unfortunately, attempts to replicated initial results have been met with high variability and limited success. At present it is not known why some studies do (Manning-Boğ et al., 2006; Kordower et al., 2006) or do not (Schapira et al., 2006; Zeng et al., 2006) find a neurotoxic effect from proteasomal inhibition.

1.4.1.4 MPTP

1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is perhaps the best-characterized PD model and has contributed significantly to treatment development (Bloem et al., 1990). MPTP is unique in that its neurotoxic effects were discovered in humans prior to the development of the animal model. In the early 1980's several cases of acutely induced, irreversible, and severe L-DOPA responsive Parkinsonism presented in young Californians following inadvertent intravenous injection MPTP (Langston et al., 1983). Neuropathological examination later revealed moderate to severe selective cell loss of dopamine neurons in the SNc, but an absence of Lewy bodies in these patients (Langston et al., 1999). MPTP crosses the blood-brain barriers and is converted, mostly in glial cells, to its toxic metabolite 1-methyl-4-phenylpridinium (MPP+) by monoamine oxidase B. MPP+ is selectively taken up in dopamine neurons due to a high affinity for the dopamine transporter (Javitch et al, 1985). MPP+ is a potent and irreversible inhibitor of complex I of the mitochondrial electron transport chain (Nicklas et al., 1985) and induces cell death via decreases in cellular adenosine-triphosphate, and free radical formation (Di Monte et al., 1986). MPTP administration induces nigrostriatal

degeneration in cats (Scheider et al., 1986) and non-human primates (Burns et al., 1983) with rats being relatively resistant to its toxic effects (Bove et al., 2005). Neurotoxic susceptibility in mice varies; with the CB57L6 strain more sensitive and Balb/C strain more resistant (Sedelis et al., 2001). As non-human primates have a motor anatomy, physiology, and repertoire very similar to humans, the clinical features of MPTP-induced nigrostriatal degeneration have been invaluable towards an understanding of PD. There are, however, two characteristics lacking in the MPTP model: pathology is restricted to the nigrostriatal pathway, and although inclusions resembling Lewy bodies have been described, inclusion formation has not been demonstrated convincingly (Forno et al., 1993; Dauer and Przedborski, 2003).

1.4.2 Genetic models

1.4.2.1 . α-Synuclein

α-Synuclein is a 140-amino acid presynaptic protein that is loosely associated with vesicles. While the exact functions of the protein are not fully understood, it is thought to be involved in vesicular handling and neurotransmitter release (Cabin et al., 2002; Yavich et al., 2004). α-Synuclein is a major component of Lewy bodies, and missense and multiplication (duplication and triplication) mutations in the α-synuclein gene have been identified in familial PD (Kruger et al., 1998; Polymeropoulos et al., 1997; Singleton et al., 2003; Chartier-Harlin et al, 2004). Several α-synuclein transgenic mouse lines have been recently generated. Ablation of α-synuclein in knockout mice appears to have little impact on neural development, is not associated with a PD

phenotype, and induces no overt signs of nigrostriatal degeneration (Specht and Schoepfer, 2001; Cabin et al, 2002, Schluter et al., 2003, Chandra et al., 2004). Overexpression of wild-type human α-synuclein is associated with widespread intraneuronal Lewy body-like inclusions in the neocortex, olfactory bulb, hippocampus and substantia nigra (Masaliah,et al., 2000). There was a slight reduction in the number putative dopaminergic striatal fibers, but no loss of dopaminergic cells in the substantia nigra and these animals exhibited only slight deficits in locomotor activity.

Perhaps surprisingly, overexpression of wild type or mutant α-synuclein in Drosophila may be the best transgenic model of progressive PD (Feany and Bender, 2000). While the nervous system develops normally in these flies, there is an age-dependent loss of a specific subpopulation of dopaminergic neurons. Furthermore, there is an age-dependent increase in α-synuclein containing inclusion formations resembling Lewy bodies. Additionally, locomotor behaviour in young flies is not altered with the mutations, but climbing ability is lost prematurely.

1.4.2.2 Parkin

Mutations in Parkin have been linked to autosomal recessive inherited PD (Abbas et al., 1999). Parkin functions as an ubiquitin-protein ligase (Zhang et al., 2000), possibly serving a role in targeting misfolded proteins for proteasomal degradation. Parkin is a known component of Lewy bodies (Shimura et al., 1999), and may be a requirement for their formation as the inclusions are notably absent in familial PD patients with parkin mutations (Dawson, 2000). A variety of different knockout mice have been created, targeting different exons of the parkin gene (Goldberg et al., 2003; ; Palacino et al., 2004;

Sato et al., 2006). While parkin deletion in these animals is associated with reductions in mitochondrial function and oxidative stress in the striatum coupled with decreased striatal dopamine release, no evidence of SNc pathology or altered behaviour is found.

1.4.2.3 DJ-1

Mutations in DJ-1 have been associated with early onset autosomal recessive PD (Bonifati et al., 2003). Chen et al., (2005) reported an age-dependent progression of reduced locomotor activity in DJ-1 knockout mice associated with neurochemical changes in the nigrostriatal pathway in the absence of nigrostriatal cell loss. In an elegant study highlighting a possible role for gene-environment interactions in PD, Kim et al. (2005) demonstrated that while DJ-1 deficiency in mice was not associated with any changes in behaviour, dopamine levels in the striatum, or SNc cell number; the null mutation conferred increased sensitivity of nigrostriatal neurons to MPTP. MPTP treatment in knockout animals was associated with greater SNc cell loss compared to wild type and, importantly, restoration of DJ-1 expression in these mice using retrograde transportation of virally introduced DJ-1 into the striatum prevented MPTP hypersensitivity.

1.5 Thesis objectives

Two studies were conducted for the present thesis. In the first study (Chapter 2), a behavioural model in the rat was developed in order to assess whether auditory cue training is associated with gross cortical neuroplasticity in the rat as measured by changes

in evoked auditory or motor evoked field responses, and prior auditory cue training can reduce haloperidol-induced akinesia in the rat under both trained and untrained motor tasks. The second study (Chapter 3) investigated whether basal-ganglia thalamocortical model assumptions of frontal cortex hypoactivity from nigrostriatal dopamine pathway degeneration would reflect in a hypotrophy of forelimb neocortical movement representations (motor maps) in the rat.

1.5.1 ***Chapter 2:*** *Sensorimotor cue training in the rat is associated with cortical neuroplasticity and can improve akinesia under haloperidol challenge.*

Rehabilitative therapies involving sensory cues have shown clinical benefit in PD (Rubinstein et al., 2002), however the mechanisms involed in cued movement facilitation are poorly understood. While it is known that some complex acoustic stimuli have an innate motorically activating capacity in akinetic rats (Whishaw, 1993; Field et al., 2000; Clark et al., 2008), it is unknown whether an initially neutral stimulus that acquires behavioural salience as a cue through associative learning can show similar behavioural activation under cataleptic challenge. The use of cue training to an initially neutral stimulus allows greater flexibility in studying cued movment facilitation as it provides assesssement of behaviour, physiology, and anatomy to the same stimulus at both baseline time points in which the stimulus does not act as a cue, and post-training when the stimulus is a cue for behaviour following associative learning.

The current study presents a model to assess sensorimotor cueing mechanisms in the rat to determine if rats could: (1) be trained to respond to an initially neutral auditory

stimulus; (2) whether cue training resulted in gross neuroplastic changes in evoked field responses in auditory or motor cortices; (2) whether prior cue training resulted in reduced akinesia during cue presentation. Rats were trained to respond to a single auditory tone or a brief train of stimulation to the medial geniculate nucleus by initiating movement to collect a sucrose pellet reward in an open recording chamber. This training task was used in order to elicit intelligent and flexible sensorimotor behaviour requiring associative learning. Neuroplastic effects of the cue training were assessed as changes in peak height of the evoked field response in auditory and motor neocortices following successful training relative to baseline. Peak height was used to provide an unbiased estimate of the magnitude of the complex evoked polysynaptic response in these pathways. In a second experiment to determine whether prior acoustic tone cue training can facilitate movement under cataleptic challenge from dopaminergic antagonism with haloperidol, performance in a probe cueing test was assessed following successful tone cue training. To determine whether cued movment facilitation could generalize to an untrained context, catalepsy in the hanging bar test was also assessed under cued conditions following successful tone cue training.

It was hypothesized that successful cue training to either auditory tone or thalamic stimulation would induces changes in the peak height of evoked auditory or motor neocortical field responses, and that prior cue training experience would lead to improved performance in both the trained and untrained beahvioural tasks under cataleptic challenge.

*1.5.2 **Chapter 3:** Neocortical movement representations are reduced and reorganized following bilateral 6-OHDA lesions of the striatum and dopamine type-2 receptor antagonism.*

The basal ganglia-thalamocortical model of PD proposes that nigrostriatal degeneration induces hypoactivity of frontal motor regions due a suppression of thalamocortical activity from excessive inhibitory basal ganglia ouput. As an index of frontal output function, unilateral 6-OHDA lesions of the medial forebrain bundle have been shown to induce subtle ipsilateral-to-lesion forelimb motor map reorganization in the rat (Metz et al., 2004) without effect on map area; however, the result of bilateral disruption of the nigrostriatal pathway on forelimb movement representations are unknown. In the present study, groups of rats received either double bilateral or unilateral left hemisphere instrastriatal 6-OHDA infusions to induce nigrostriatal degeneration and underwent high-resolution intracortical microstimulation (ICMS) electrophysiological mapping of the left sensorimotor cortex two weeks later to assesss forelimb motor map expression. Assays of map expression consisted of three related components: (1) map size - the total area of the cortex able to elicit stimulation-induced forelimb movments; (2) map organization - the distribution ratio of proximal (shoulder and elbow) and distal (wrist and digit) movement representations; and (3) map excitability – the minimum current intensity threshold to induce forelimb movements. Nigrostriatal degeneration induced by 6-OHDA infusion was quantified by assessing tyrosine hydroxylase (TH) immunoreactivity in the SNc. As sensorimotor impairments resulting from the the lesions may themselves alter map expression, a second study derived forelimb motor maps under

acute dopaminergic antagonism with systemic haloperidol to ensure that dopamine antagonism alone is sufficient to induce alterations in map expression.

It was hypothesized that bilateral dopamine antagonism from intrastriatal 6-OHDA infusion and systemic haloperidol would lead to significant reduction in forelimb map area, increased thresholds to evoke forelimb movements, and a reorganization in the distribution of proximal (shoulder and elbow) and distal (wrist and digit) movment representations. Unilateral instrastriatal 6-OHDA infusion was hypothesized to result in forelimb map reorganization without effect on map area or thresholds.

Chapter 2

Sensorimotor cue training in the rat is associated with cortical neuroplasticity and can improve akinesia under haloperidol challenge

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Running title: **Sensorimotor Cue Training**

First Author Contribution:
Electrode implantation surgery, behavioural training and testing, electrophysiology, data analysis, manuscript preparation

Key words: Sensory cueing, movement facilitation, Parkinson's disease, evoked response

2.0 Abstract

Sensory cues can improve movement deficits in Parkinson's disease, but little is known on the mechanisms involved. A behavioural model in the rat in which to assess sensorimotor cueing behaviours was addressed in the present study. Rats were trained to respond to auditory tone or thalamic stimulation cues by retrieving a food reward. Following successful training, changes in thalamo-auditory and auditory-motor evoked field responses were assessed. Tone cue-trained rats were then tested for cued movement facilitation under haloperidol. Auditory tone cue training was associated with a significant increase in peak height of the evoked thalamo-auditory, but not auditory-motor response. Successful cue training was observed with thalamic stimulation cues, but was without effect on either evoked response. Tone cue training improved movement facilitation under haloperidol challenge in task dependent manner. Results indicate that auditory tone cue training induces neuroplastic changes in auditory neocortex which may be related to improved sensorimotor function under dopaminergic antagonism.

2.1 Introduction

Core hypokinetic symptoms in Parkinson's disease present as difficulty in the initiation (akinesia) and poverty and slowness in the execution (bradykinesia) of movement. It has long been known that PD patients, despite severe hypokinesia, are able to perform movements in response to external sensory cues with greater velocity, force, and accuracy than they would otherwise be able to voluntarily (Schwab, 1959; Martin, 1967; Forssberg et al., 1984). This "Paradoxical kinesia" in PD suggests that while motor function capacity is preserved, movement deficits occur as a result of impaired motor control (Rubinstein et al., 2002). In recent years, renewed interest in cued movement facilitation in PD has been focused on developing novel rehabilitation therapies that employ auditory cues as adjunctive therapies to pharmacotherapy (Thaut, et al., 1996; Thaut et al., 2001; Rubinsteain et al., 2002; Howe et al., 2003; Dibble et al., 2004; ; Ma et al, 2004; Suteerawattananon et al., 2004).

Little is known about the recruitment mechanisms by which auditory cues can bypass faulty internal cue and pre-movement set related activity of the basal ganglia in PD (Morris and Iansek, 1996), but this understanding is of great clinical interest in order to focus and maximize the effects of rehabilitative therapies. Rat models provide and excellent substrate in which to answer these questions by providing unparalleled flexibility and amenability to numerous experimental interventions compared to human studies (Cenci et al., 2002). The auditory (Weinberger and Diamond, 1987; Bao et al., 2004; Polley et al., 2006) and motor (Kleim et at., 1998; Kleim et al., 2002; Kleim et al., 2003; Kleim et al., 2004) neocortices of the rat have shown to be highly malleable,

demonstrating anatomical and physiological neuroplastic phenomena induced by behavioural learning. Importantly, reduced dopaminergic function in the rat by either pharmacological antagonism (Sanberg, 1980; Lorenc-Koci et al., 1996) or neurotoxic lesions of the nigrostriatal dopamine system (Deumens et al., 2002) provide a model of PD demonstrating the majority of human symptomology (Cenci et al., 2001; Deumens at al., 2001). Moreover, findings that auditory stimuli have been shown to induce a release from catalepsy and reinstatement of previously lost behaviour due to brain injury in the rat (Whishaw, 1993; Field et al., 2000; Clark et al., 2008), resemble auditory cue facilitation of movement in PD (Oliveira et al., 1997; Ma et al., 2004).

The current study presents a behavioral model to assess auditory-stimulation induced cortical plasticity in the rat. Rats were trained to respond to a single auditory tone or a brief train of stimulation to the medial geniculate nucleus by initiating movement to collect a sucrose pellet reward in an open recording chamber. Following successful training, neuroplastic effects of the training was assessed as changes in peak height of the evoked field response in auditory and motor neocortices. In a second experiment to determine whether prior acoustic tone cue training can facilitate movement under antagonism of dopaminergic systems, performance in a probe cueing test and the hanging bar task was assessed under haloperidol cataleptic challenge.

2.2 Experiment I:

Auditory tone cue training is associated with cortical neuroplasticity

2.2.1 Methods

2.2.1.1 Ethical considerations

All procedures involving rat use in this study strictly adhered to the guidelines of the Canadian Council on Animal care and were approved by the Institutional Animal Care committee of the University of Calgary. All efforts were made adhere to the principles of reduction, refinement, and replacement in experimental design (Russell & Burch, 1959), with every attempt made to limit the number of subjects and minimize animal suffering.

2.2.1.2 Rats

A total of 25 adult male Long-Evans rats weighing 254-392 g at the time of electrode implantations were used in this study. All rats were obtained from the University of Calgary Breeding Colony and housed individually in clear plastic cages in a colony room maintained on a 12h light/dark cycle (lights on at 07:15) at 21°C. Rats were provided free access to food and water throughout the duration of their housing except for being food restricted to 90% of their implantation body weight at electrode implantation during behavioural training.

2.2.1.3 Experimental groups and procedures

Rats were randomly assigned into four groups: tone cue-trained (n = 8), non tone cue-trained (n = 8), stimulation cue-trained (n = 6), and non stimulation cue-trained (n = 5). Reported sample sizes were unequal between groups due to surgical and head cap loss

of rats (n = 2 for stimulation cue-trained, n = 3 for non stimulation cue-trained) Seven days following implantation surgery, baseline electrophysiological responses (described below) were taken for each rat. Rats then underwent behavioural training until criterion was reached (described under the behavioural training section), and follow-up electrophysiological records obtained.

2.2.1.4 Chronic electrode implantation

Rats were anaesthetized with isoflurane (4% induction, 1.5% maintenance; VIP-3000 Vaporizer, Matrix, Orchard Park, NY) and placed in a stereotaxic frame with the incisor bar set to skull flat. The local anaesthetic lidocaine (2%) was administered subcutaneously at the incision site and the scalp incised. Twisted-wire bipolar recording and stimulating electrodes were constructed from Teflon-coated stainless steel wire 178 μm in diameter (A-M Systems, Everett, WA). Electrode terminals were connected to gold-plated male amphenol pins and the two uninsulated poles for implantation were separated by 0.5 mm.

Three bipolar electrodes were chronically implanted in the right hemisphere according to the stereotaxic co-ordinates of Paxinos and Watson (1986) at the following coordinates relative to bregma: primary auditory field (Te1; AP -5.8, ML + 6.0, V −4.3 mm), primary sensorimotor cortex (SM1; AP +1.0, ML + 4.0, V −2.5 mm), ventral division of the medial geniculate nucleus (MGv; AP -5.8, ML + 3.7, V −5.8 mm). The ventral division of the medial geniculate nucleus was chosen as the stimulation site as it is the sole division of the medial geniculate that comprises the lemniscal pathway conveying only auditory information tonotopically organized, to the primary auditory

field (Romanskey et al., 1993). The caudal region of the primary auditory field was chosen for the recording site as low frequencies are represented in this area (Sally and Kelly, 1988; Scheel, 1988) and a low frequency, 1 kHz tone was utilized in the experiment. Somatosensory recording coordinates were chosen as they corresponded to the output layer of the forelimb region of the primary motor cortex (Rouiller et al., 1993; Teskey et al., 2002).

Electrophysiological monitoring was performed during surgery and dorsal-ventral placements of the electrodes adjusted to maximize the amplitude of the evoked responses. The amphenol pins connected to the electrodes were then inserted into a nine-pin McIntyre connector plug (Molino & McIntyre, 1972; Ginder Science, Ottawa, ON) which was adhered to the skill with dental cement and anchored with five stainless steel screws, one of the screws serving as a ground electrode. The scalp was then sutured around the headcap comprising the dental cement and plug, and rats were given a topical application of Xylocaine jelly (2%) anaesthetic around the incision. Subjects were monitored continuously throughout the surgery and anaesthesia levels adjusted as needed to maintain surgical depth. All subjects were given seven days to recuperate prior to obtaining baseline electrophysiological records

2.2.1.5 Evoked field potentials

Evoked potentials were recorded as per Flynn and Teskey (2007). Input/Output records were obtained by administering pulses of increasing intensity to MGv and Te1, separately, with the resultant evoked field potentials recorded in primary auditory (MGv stimulation) and motor (Te1 stimulation) neocortical electrodes recorded. Current

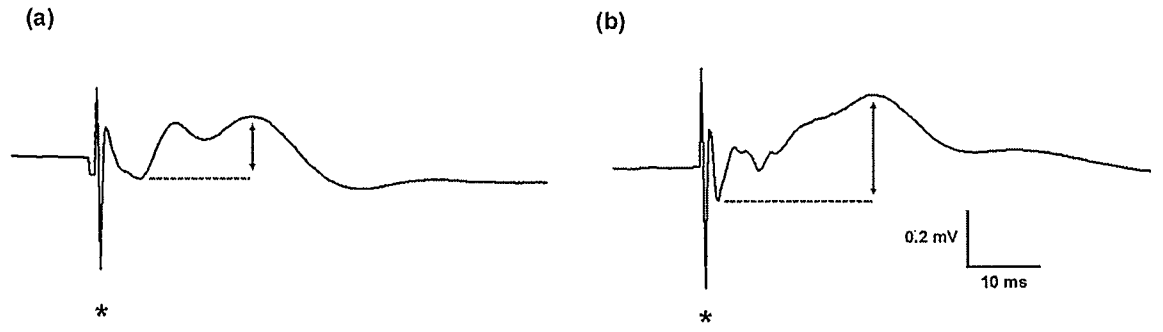
consisted of biphasic rectangular pulses with a width of 0.2 ms and a 0.2 ms delay between phase inversions. Ten intensities were used (32, 46, 68, 100, 147, 215, 316, 464, 681, and 1000 μA) and ten pulses at a frequency of 0.1 Hz were given at each intensity for a total of 100 pulses. Stimulation voltages were computer generated and then amperage converted by use of a constant current isolation unit (World Precision Instruments, Sarasota, FL, USA). The recorded signals were filtered at half amplitude below 1 Hz and above 100 Hz and amplified by 1000 (Grass Neurodata Acquisition System Model 12). Analog signals were digitized at a sampling rate of five points per ms and a mean of the 10 single pulses were calculated for each of the intensities using the SciWorks data acquisition software (Datawave, Longmont, CO, USA).

2.2.1.6 Evoked potential analysis

Evoked potentials obtained prior to and following cue training were examined for changes in the relative peak height of the response. Relative peak height was used as it provided a relatively unbiased quantification of the complex, polysynaptic evoked responses and was defined as the smallest, trough, value (mV) of the response subtracted from the largest, peak, value (Figure 2-1). Records at a stimulation intensity of 464 μA were used for statistical analyses as this stimulation intensity resulting in maximum amplitude of the evoked response. Any cases where a clear electrophysiological response could not be ascertained due to noise or instability in the I/O series were excluded from the analyses. For analyses, the percent change in the peak height of the response post training to baseline was used to compare groups. Percent change in peak height was

Figure 2-1: Sample recorded field potentials evoked with 200 μS biphasic square wave pulses at an intensity of 464 μA. (a) Response in the primary auditory cortex (Te1) from stimulation of the ventral division of the medial geniculate (MGv). (b) Response in the primary sensorimotor cortex (SM1) from stimulation of the primary auditory cortex (Te1). Arrows indicate peak height of the response. Stars indicate stimulation onset.

Figure 2-1: Sample evoked feild responses



calculated as (peak height post training – peak height at baseline) / (peak height at baseline)*100.

2.2.1.7 Behavioural training

Cue group rats were trained to retrieve one-gram sucrose reward pellets (Bioserve, Frenchtown, NJ) upon cue presentation in an open recording chamber. Cues were either a 5-second presentation of a 1 kH pure tone sinusoidal waveform (tone cue; 70 – 80 db SPL; Audacity v1.3) or a 5-second train of biphasic stimulation of the ventral division of the medial geniculate (stimulation cue). Thalamic stimulation consisted of biphasic rectangular pulse trains with a 0.2 ms pulse with and 0.2 ms delay between phase inversions at an intensity of 1 mA and a frequency of 10 Hz.

Training consisted of three distinct stages. Cue rats were first acclimatized to the recording chamber (32 x 57 cm) and provided free access to the pellet rewards placed in a 4 x 4 cm (VWR International) polystyrene disk for fifteen minutes each day for two consecutive days. After acclimatization, cue group rats were slowly shaped to respond to the cue by initiating movement to retrieve the sucrose pellet placed in the weigh dish. The rat was placed at the starting location (rear-right of chamber) and the cue given. If the rat did not initiate movement to retrieve the pellet by the end of the stimulation, a pellet was delivered to the rat by the experimenter with forceps. This was done to prevent any extinction during training. If the rat attempted to retrieve the pellet prior to the cue, the weight dish containing the pellet was removed from chamber in a line on the diagonal from the start-point and the left-front corner of the chamber and the trial was restarted. As shaping continued, the weigh dish was slowly moved further away from the starting

position in 1.0 cm increments following each successful trial. To facilitate the rate of shaping, a second pellet was placed at the starting location after each trial to train rats to return to the starting location at the end of a trial. One session was conducted every day and consisted of twenty trials. Once rats could complete two consecutive successful trials when the weigh dish was placed at distance of 10 cm from the start location, the third training phase commenced. In this phase, the pellet was placed in the weight disk (45 cm) from the start point, and one session of 20 trials conduced each day with the number of successful trials recorded. Training was continued until rats were able to complete 18 out of 20 successful trials in a day.

Respective (tone and stimulation) control rats were yoked to their experimental counterparts so that for each cue-trained rat there was a control rat who received the same number of sessions (cue and pellet presentations) as their experimental partner. For control rats, a session consisted of a fifteen-minute period in which the cue stimulus was delivered non-contingently with food reward for a total of 20 presentations. During the session, rats were allowed free access to food pellets placed in the weight dish in the same location as stage three for experimental rats. Once a rat retrieved a pellet and returned to the start location, another was placed in the dish using forceps. Following successful training for experimental rats (nine days for stimulation cue and five days for auditory cue groups) and equivalent sessions for their yoked controls, follow-up input-output responses were taken for each rat.

2.2.1.8 Statistics

Statistical analysis were conducted using the SPSS 16.0 (SPSS Inc., Chicago, IL)

Two-tailed Student *t*-tests were to assess group difference in evoked potential measures and the number of trials required to reach training criterion between tone and stimulation cue conditions. An *a priori* significance level of .05 was used for all analyses. All data are presented as mean ± SEM.

2.2.2 Results

2.2.2.1 Behavioural training results

There was a significant difference between tone and stimulation cue groups in the number of trials required to reach behavioural training criterion (t_{11} = 8.328, $P < .001$). Tone cue rats required significantly fewer (155 ± 8) trials than stimulation cue rats (272 ± 12) to reach criterion.

2.2.2.2 Evoked potentials

Group evoked potential data is summarized in Figure 2-2. For the tone cue condition, a significant difference was observed between tone cue trained and tone cue control rats (t_{13} = 2.736, P <.015) in the percent change of the peak height of the geniculo-auditory pathway. Tone cue-trained rats had a significant increase (30.32 ± 14.62%) in peak height of this pathway following training compared to control rats (-19.89 ± 11.46). No differences (t_8 = 0.822, P = .435) were observed between tone cue trained (57.96 ± 35.08%) and tone cue control (15.07 ± 35.78) rats in the percent change of the peak height of the auditory-motor pathway. For the stimulation cue condition, no differences (t_7 = 0.374, P = .720) were observed between stimulation cue trained (-13.07

± 17.16%) and stimulation cue control (-5.38 ± 12.35) rats in the geniculo-auditory pathway. Similarly, no differences were observed (t_3 = 0.315, P = .757) between stimulation cue trained (-24.08 ± 21.75%) and stimulation cue control (-14.22 ± 9.90) rats in the auditory-motor pathway.

2.3 Experiment II:

Prior auditory tone cue-training improves trained task performance under haloperidol

2.3.1 Methods

2.3.1.1 Rats

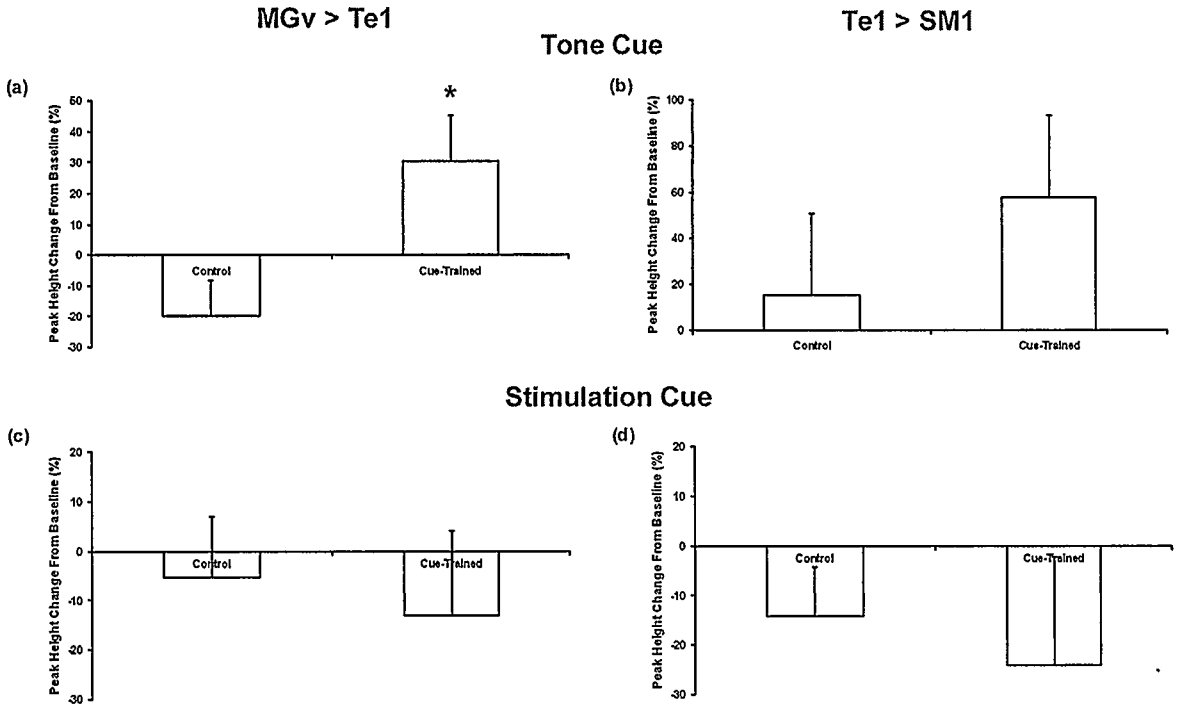
Subjects were 16 adult, male Long-Evans rats from tone-cue and tone-cue control groups in experiment I weighing 328-401 g at the time of behavioural testing. Rats were housed in the same manner as in experiment I.

2.3.1.2 Experimental groups and procedures

Rats had been previously randomly assigned into two groups in experiment I: tone cue-trained (n=8) and non tone cue-trained (n=8). Eight days following post-training electrophysiological recordings, a second cueing test was performed to determine whether cue trained rats were able to perform the task at this time point. The following day rats underwent testing under vehicle control drug conditions in a probe cueing task

Figure 2-2: Percent change in relative peak height (mean ± SEM) of evoked auditory (MGv > Te1; a,c) and sensorimotor (Te1 > SM1; b,d) field responses in tone cue (top panel) and thalamic stimulation cue (bottom panel) groups following cue training relative to baseline. Peak height was calculated as the smallest trough value subtracted from the largest peak (mV). Abbreviations: MGv, ventral division of the medial geniculate nucleus; Te1, primary auditory cortex; SM1, primary somatosensory cortex. Means marked with a star are significantly different from their corresponding control mean ($p < .05$).

Figure 2-2: Changes in peak height of evoked auditory and sensorimotor responses



(described below) and the hanging bar test. Rats were then retested the next day on these tasks under haloperidol challenge.

2.3.1.3 Drugs

Haloperidol (Sigma-Aldrich, St. Louis, MO) was dissolved in a 10% acetic acid solution, the pH adjusted to 6 with NaOH (Håkansson et al., 2005), and brought to a final concentration of 0.5 mg/ml with 0.9 % saline.

2.3.1.4 Post-training behavioural challenges

Eight days after follow-up electrophysiological measures, a behavioral assessment of tone cue-trained and non tone cue-trained rats was undertaken to investigate any effects of prior cue training on movement initiation under cataleptic haloperidol challenge. To ensure that trained rats were able to complete the cueing task at this time point, a ten-trial test was conducted using the same methods as the third phase of behavioural training and confirmed maintenance in performance at this time point (presented in results) For the cueing task, rats were placed in the rear right start position of the chamber, and a pellet-baited weigh boat placed 45 cm away on the diagonal between the start position and the front left corner of the chamber. A trial was considered successful if the rat initiated movement towards the pellet from the time of the auditory cue initiation to its termination five seconds later and the rat successfully retrieved the pellet.

On the following day, baseline measures in probe cueing test and hanging bar task were assessed for both groups under vehicle conditions, with the tests being repeated the

next day under haloperidol cataleptic challenge (1 mg/kg i.p., behavioural testing conducted 90 minutes following injection). This dose of haloperidol was shown in preliminary investigation to instill a marked decrease, but not abolishment, of spontaneous locomotor activity in rats at this time point. The order of the tests on each experimental day was counterbalanced between rats. As a metric to assess whether prior cue training improved performance in the probe cueing task under haloperidol challenge, the percent change in error rates between haloperidol and vehicle drug conditions was calculated as 1 – (# successful trials under haloperidol / # successful trials under vehicle) * 100. Using this measure, a 0% change in error rate indicates no impairment in task performance under haloperidol, whereas a 100% change error rate indicates that rats can not perform the task under haloperidol challenge. The hanging bar test (Sanberg et al., 1988) was used to assess whether prior auditory cue training can reduce haloperidol induced akinesia, assessed using decent latency (s) to withraw a forepaw, during cue presentation in an untrained task.

Following post-training electrophysiological measures, thalamic stimulation cue groups were deeply anaesthetized with sodium pentobarbital and transcardially perfused with cold physiological saline. The brains were removed and immersed whole in 20 ml of Golgi-Cox solution for 12 days. The brains were then cryoprotected and sectioned according to Miklyaeva et al., (2007). Assessment of dendritic branch length (Sholl, 1956) and spine density (Woolley, 1990) was assessed in layer III of primary auditory and motor cortices as per Miklyaeva et al. (2007) (not reported).

2.3.1.5 Probe cueing task

Behavioural assessment included a probe cueing task from experiment I and the hanging bar test. In the probe cueing task, rats were placed in the starting position of the recording chamber with a pellet reward placed in the weight boat 5 cm from the rat. Upon tone cue presentation (1 kHz sine wave for 5 seconds, 70-80 dB SPL), rats were allowed to retrieve the pellet. A successful trial was defined as when the rat initiated movement to succesffuly retrieve the pellet prior to cue termination (5 s). Ten consecutive trials were performed for this task. As non cue-trained rats were expetedly found to not be able to perform the cueing test from Experiment I (shown in results), the cueing probe task was conducted in this fashion in order to allow baseline performance in non cue-trained rats amenable for comparison with cue-trained rats.

2.3.1.6 Hanging bar test

The hanging bar test (Sanberg et al., 1988) consisted of a stainless steel bar (1 cm diameter, 30 cm length) suspended horizontally 10 cm from the ground by support beams. The forepaws of the rat were placed on the bar 5 cm apart and the tone cue was immediately given (time 0). The latency (s) for at least one forepaw to be released from the bar was recorded (descent latency).

2.3.1.7 Statistics

Statistical analysis were conducted using the SPSS 16.0 (SPSS Inc., Chicago, IL) Data assessing performance in the probe cueing task under haloperidol and vehicle conditions were not normally distributed according to the Wilks-Shapiro test ($P < .05$)

and were assessed using the Mann-Whitney U test. The percent change in the number of successful trials in the probe cueing task under haloperidol compared to vehicle was used to create an index score of movement impairment according to the following formula: 1 – (# successful trials under haloperidol/ # successful trials under vehicle)* 100. Performance in the hanging bar test was calculated as descent latency under the haloperidol condition subtracted from the descent latency under vehicle. The percent impairment in the probe cueing task was analysed with a one-tailed student *t*-test. Paired sample two-tailed *t*-tests were used to compare descent latencies in the hanging bar test within groups between vehicle and haloperidol conditions. Two-tailed Student *t*-tests were used to assess group difference in descent latencies. When assumptions of variance homogeneity were violated, Welch's protected t-tests with corrected degrees of freedom were used. An *a priori* significance level of .05 was used for all analyses. All data are presented as mean ± SEM.

2.3.2 Results

2.3.2.1 Cueing task performance

To ensure that rats were still able to perform the cueing task after follow-up evoked potentials were recorded; a 10-trial cueing test with the same criteria as in Experiment I was conducted for auditory cue trained and auditory cue control rats. Tone cue-trained rats scored 9.5 ± 0.3 / 10 correct trials compared to 0.25 ± 0.3 for non tone cue-trained rats. This difference was significant ($Uz = 3.416$, $P < .001$) and indicated that cue-trained rats could still perform the task at this time point 16 days following training.

Tested under the probe cueing task, tone cue-trained rats scored 9.9 ± 0.2 correct trials out of 10 under vehicle and 5.6 ± 1.3 under haloperidol. Non tone cue-trained rats scored 4.4 ± 1.4 correct trials out of 10 under vehicle and 1.4 ± 0.3 under haloperidol. As a metric assessing When the percent change in error rates in the probe cueing under haloperidol and vehicle was compared between groups (Figure 3-3), cue-trained rats exhibited significantly (t_{13} = 1.79, P = .048) less impairment (61 ± 11.5 %) compared to non cue-trained rats (31.4 ± 14.6).

2.3.2.2 Hanging bar test performance

In the hanging bar test, mean descent latency was significantly greater under haloperidol compared to vehicle in both cue-trained and non cue-trained groups. Cue-trained rats exhibited a mean descent latency in the no cue condition of 23.38 ± 6.38 s under haloperidol and 0.53 ± 0.06 s under vehicle. These values significantly differed (t_7 = 3.590, P = .009). In the cued condition, cue-trained rats exhibited a similar significant (t_7 = 3.769, P = .007) increase in mean descent latency under haloperidol (22.32 ± 5.81 s) compared to vehicle (0.536 ± 0.06 s) conditions. Non cue-trained rats exhibited a mean descent latency in the no cue condition of 14.10 ± 1.97 s under haloperidol and 0.47 ± 0.07 s under vehicle. These values significantly differed (t_7 = 6.886, P < .001). In the cued condition, non cue-trained rats exhibited a similar significant (t_7 = 5.135, P = .001) increase in mean descent latency under haloperidol (18.79 ± 3.60 s) compared to vehicle (0.49 ± 0.06 s) conditions.

When assessing a training effect on descent latencies, no differences were observed between cue-trained and cue-control groups under haloperidol in either cue (t_{14} = -.575, P

= .574) or no cue ($t_{8,317}$ = 1.388, P = .201) conditions. In the cue condition, cue-trained rats exhibited a mean descent latency of of 22.32 ± 5.81 s under haloperidol compared to 18.79 ± 3.60 s for cue-controls (Fig. 2-4). In the no cue condition, cue-trained rats exhibited a mean descent latency of 23.38 ± 6.38 s of under haloperidol compared to 14.10 ± 1.97 s for non cue-trained controls (Fig. 2-4).

Figure 2-3: Percent change in error rates in the probe cueing task (mean ± SEM) under haloperidol (0.5 mg/kg i.p., 90 minutes prior to testing) relative to vehicle control drug conditions between auditory tone cue-trained and non cue-trained groups. Increased values of changes in error rates represents decreased task performance measured as the number of correct trials (/10) to initiate movment within 5 seconds of a 1 kHz 5 second auditory tone presentation to successfully retrieve a food pellet reward at a distance of 5 cm from the rat. Mean marked with a star significantly differed from corresponding control mean ($P < .05$).

Figure 2-3: Probe cueing task performance under haloperidol

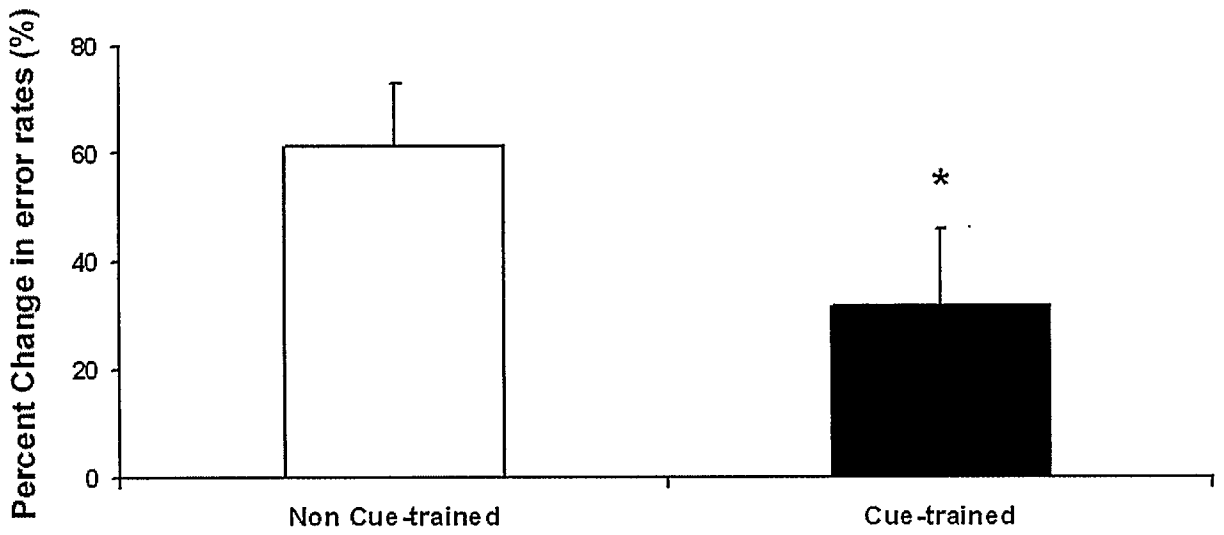
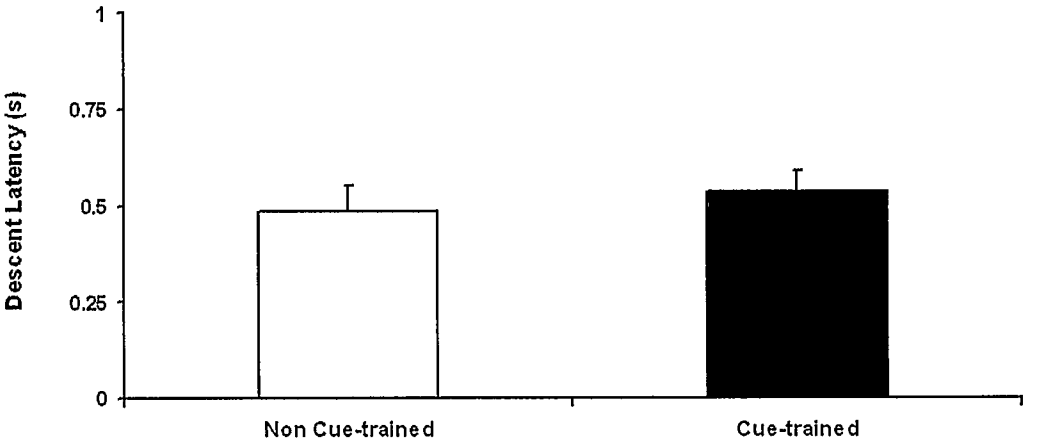


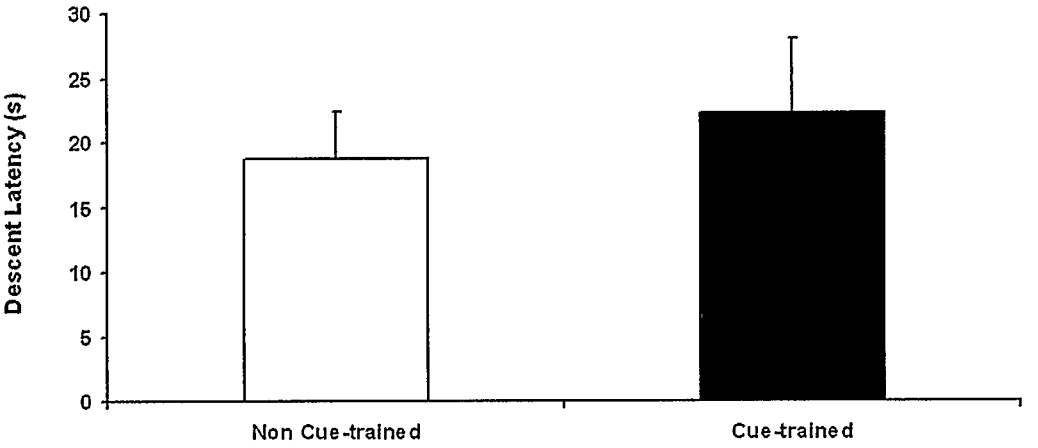
Figure 2-4: Descent latencies in the hanging bar test (mean ± SEM) to a 1 kHz 5 second auditory tone presentation for cue trained and cue control rat under (a) vehicle control and (b) haloperidol (0.5 mg/kg i.p., 90 minutes prior to testing). Descent latency was recorded as the latency (s) for at least one forepaw to be released from the bar relative to the simultaneous placement of the rat in the apparatus and the start of the tone presentation. A marked increase in descent latency is observed under haloperidol compared to vehicle control drug conditions. No significant differences were observed under either drug condition between prior auditory cue training and cue training control groups ($P > .05$).

Figure 2-4: Hanging bar test descent latencies under haloperidol

(a)



(b)



2.4 Discussion

Primary results of this study indicate that associative auditory cue training in the rat induces changes in thalamocortical evoked responses and facilitates behavioural release from cataleptic challenge with haloperidol. Rats were trained to respond to an auditory tone cue by initiating movement to collect a food reward, and training resulted in a significant increase in the peak height of the evoked auditory cortex field response to stimulation of the medial geniculate nucleus. In a second experiment, prior auditory cue training was shown to improve cued behavioural activation under haloperidol as indicated by increased performance in trained rats to initiate movement to collect a food reward relative to rats that did not receive training.

It is becoming increasingly apparent that learning can induce plastic changes in a wide variety of cortical areas (Scheich et al., 1997; Kleim et al., 1998; Buonomano and Weinberger, 1998; Edeline, 1999). In the auditory cortex, receptive field plasticity can be induced by both appetitive (Kisley and Gerstein, 2001; Bao et al., 2004; Polley et al., 2006; Hui et al., 2009) and aversive associative learning (Bakin and Weinberger, 1990; Edeline and Weinberger, 1993; Bakin and Weinberger, 1996; Gao and Suga, 1998). Perceptual auditory training is associated with a reorganization of cortical auditory map representations in that increased cortical responses are observed to trained stimulus frequency and intensity ranges (Bao et al., 2004; Polley et al., 2006). When pure tones are used in conditioned learning paradigms, enhancement of cortical responses near target frequencies and reduced responses to non-reinforced frequencies are observed (Ohl and Scheich, 1996; Blake et al., 2002). Present results of tone cue-training induced changes of

primary auditory cortex excitability agree with findings that both pure tones (Ohl et al., 1996) and simple behavioural training (Boa et al., 2004) can alter auditory cortical processing. It is perhaps unsurprising then that thalamic stimulation cue training was unassociated with changes in neocortical evoked responses in the present study. While auditory tone cue training involved a discrete, pure tone stimulus, thalamic stimulation may be less specific resulting in no change in frequency tuning curves and excitability of auditory cortex neurons. This may be supported by the finding that auditory tone training was associated with a more rapid rate of task acquisition than thalamic stimulation training, suggesting a lower salience of thalamic stimulation to auditory tones which would be expected if the cue was less specific.

Auditory tone cue training was not associated with neuroplastic changes in the auditory-motor pathway. This finding should be interpreted with caution, however, as an increased mean in cue-trained compared to non cue-trained rats was observed in addition to a large variance associated with this measure. Therefore, an increased sample size may change the present findings. Changes in evoked intracortical responses in primary motor cortex were assessed as this area serves as the output of the pyramidal motor system. Changes in corticospinal output were of interest in relation to the activation effects of prior cue training experience to tone presentation under cataleptic challenge. It is known that auditory activation of motor-related structures are observed in secondary (lateral premotor, supplementary motor) and associational (posterior parietal) motor areas (Schubotz et al., 2000; Rauschecker, 2001; Popescu et al., 2004) more so than primary motor cortex. That these areas are also noted to be hyperactive in PD (Hanakawa, Katsumi, et al., 1999; Hanakawa, Fukuyuma, et al., 1999, Shibasaki et al., 2004) may

reflect increased recruitment of external sensorimotor mechanisms to compensate for damaged basal ganglia-thalamocortical circuitry (DeLong and Whichmann, 1993; Morris and Iansek, 1996; Morris et al., 1996), and suggests that cue training-induced plastic changes may be observed in secondary motor areas in the present experiment. However, a dissociation between cue training induced neuroplasticity and behaviour was observed here in that thalamic stimulation cue-trained rats learned the task without demonstrating any change in evoked cortical responses. Therefore, the functional nature of enhanced evoked responses in auditory cortex observed in the present study, or similar unseen changes in other cortical areas, in relation to motor performance in the task is unknown.

In the second experiment of this study, it was shown that prior auditory tone cue training resulted in a significant increase in performance in the probe cueing task under haloperidol cataleptic challenge relative to rats that did not receive tone cue training. This finding is reminiscent of paradoxical kinesia in PD (Rubinstein et al., 2002). Tone cue training effects under haloperidol appear to be specific to the trained motor task as prior cue training experience was not shown to affect descent latency in the hanging bar test compared to rats that did receive cue training. Haloperidol is a D2 receptor antagonist and these results further support that expression of learned motor skills is resistant to D2 receptor antagonism (Levesque et al., 2007; Tremblay et al., 2009). As impaired motor learning has also been observed in PD (Doyon et al., 1997; Doyon et al., 1998; Shin and Ivry, 2003; Siegert et al., 2006), rehabilitative therapies incorporating a focus on previously learned motor skills may be of benefit. It seems unlikely that increased performance in cue trained rats under haloperidol may be due to increased dopamine release from the auditory cue predicting the food reward or the visual stimulus of the

reward itself (Schultz, 2000) as it has been reported that paradoxical kinesia in the rat is not a consequence of dopamine release (Keefe et al., 1989). However, task retention in trained rats could be due to a pre-training (Caldji and Vanderwolf, 1996) effect in which the expression of previously learned behaviours are not affected by dopaminergic antagonism.

In summary, pr esent results support the notion that auditory tone cue training induces neuroplastic changes in auditory neocortex which may be related to improved sensorimotor function under dopaminergic antagonism. While these findings share commonalities to features of paradoxical kinesia; results were found to be dependent on prior cue training experience, and were specific to the cue-training task. It is known that some complex acoustic stimuli have an innate motorically activating effect in akinetic rats (Whishaw, 1993; Field et al., 2000; Clark et al., 2008). As rehabilitative therapies involving sensorimotor cueing have shown clinical benefit in PD (Rubinstein et al., 2002), further investigation into the mechanisms involved in trained and untrained motor facilitation from sensory stimulation seem worthwhile.

Chapter 3

Neocortical movement representations are reduced and reorganized following bilateral 6-OHDA lesions of the striatum and dopamine type-2 receptor antagonism.

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3.0 Abstract

The neurophysiological model of Parkinson's disease predicts nigrostriatal degeneration leads to increased inhibitory output of the basal ganglia and hypoactivation of the frontal neocortex. The nature of this frontal hypoactivation is not well understood. Intracortical microstimulation (ICMS) was used to probe topographic movement representations of the left forelimb neocortical motor area in rats two weeks following sham, unilateral left hemisphere or bilateral intrastriatal 6-hydroxydopamine (6-OHDA) lesions or under acute dopamine receptor antagonism with haloperidol. 6-OHDA lesions were characterized by a significant loss of substantia nigra dopamine neurons in lesion relative to non-lesion hemispheres. Bilateral 6-OHDA lesions or haloperidol administration significantly reduced forelimb map areas. However, both bilateral and unilateral lesions resulted in significant map reorganization characterized by an increase in the proportion of distal-to-proximal movement representations. Results suggest dopamine deficiency in the basal ganglia can affect the topographic organization of sensorimotor neocortex and lead to significant reduction of motor representation in the forelimb area when the lesion is bilateral.

3.1 Introduction

Degeneration of the nigrostriatal dopamine system is a hallmark of Parkinson's disease (PD). According to the basal ganglia-thalamocortical circuit model, striatal dopamine depletion results in excessive inhibitory activities in the basal ganglia output nuclei leading to increased inhibition of thalamocortical projections and, in turn, a suppression of activity in the frontal neocortex underlying hypokinetic motor symptoms (Alexander et al., 1990; DeLong and Wichmann, 1993; Parent and Hazrati, 1995; Wichmann and& DeLong, 2003). Nigrostriatal degeneration resulting in sensorimotor behavioural impairments in the rat can be induced by 6-hydroxydopamine (6-OHDA; Deumens, et al., 2002). Supporting basal ganglia-thalamocortical circuit theory predictions, a reduction of metabolic activity in lateral thalamic neurons and in frontal cortical areas including the primary motor cortex has been reported in 6-OHDA lesioned rats (Rolland et al., 2007). 6-OHDA lesion also reduces the immediate early gene expression in this brain region (Steiner and Kitai, 2000). Neuroimaging studies in PD patients off their dopaminergic mediation reveal impaired movement-related activation in some, but not all, frontal motor areas (Jenkins et al., 1992; Playford et al., 1992; Rascol et al., 1992; Rascol et al., 1994; Sabatini et al., 2000; Haslinger et al., 2001; Buhmann et al, 2003). This impairment is at least partially reversed with dopaminergic therapy (Jenkins et al., 1992; Rascol et al., 1992; Haslinger et al., 2001; Buhmann et al, 2003) supporting the notion of that nigrostriatal degeneration induces functional deafferentation of the frontal cortex. On the other hand, recent studies in monkeys indicate that the current model of PD may be too simplistic as lesion or stimulation placed in the internal pallidal

segment of the basal ganglia induced motor response deficits inconsistent with an inhibitory connection between the basal ganglia and motor thalamus (Anderson et al., 2003; Desmurget and Turner, 2008).

The sensorimotor neocortex of the rat is an ideal system to investigate detailed alterations following nigrostriatal lesions. Forelimb neocortical regions are known to serve as an output of the basal ganglia-thalamocortical circuit (Rouiller et al., 1993). Motor map expression in the rat can be revealed by intracortical microstimulation (ICSM), which has been shown to be sensitive to the influence of both brain injury and experience (Piecharka et al., 1995; Kleim et al., 2003; Williams et al., 2006). Finally, in this brain region, synaptic potentiation and depotentiation phenomena can be precisely correlated with both anatomical and physiological changes in neocortical movement representations (motor maps; Teskey et al., 2002; Teskey & Monfils, 2004; Monfils et al., 2005; Teskey et al., 2007; Teskey et al., 2008).

In a previous study it was found that forelimb map expression was preserved following unilateral 6-OHDA lesions of the medial forebrain bundle despite a marked motor impairment in lesioned rats (Metz et al., 2004). However, motor map expression has not been examined with intrastriatal 6-OHDA lesions, a model thought to more closely approximate nigrostriatal degeneration in PD (McGeer et al., 1998; Deumens et al., 2002). In this study, groups of rats were given either bilateral, left hemisphere unilateral or sham 6-OHDA infusions and underwent high-resolution ICMS mapping of the left neocortical forelimb area two weeks post-lesion. Nigrostriatal degeneration was quantified by assessing tyrosine hydroxylase (TH) immunoreactivity in the substantia nigra pars compacta (SNc). A second experiment investigated acute dopaminergic

antagonism on map expression with forelimb motor maps derived by ICMS under systemic haloperidol.

3.2 Experiment I:

Intrastriatal 6-OHDA infusions on expression of neocortical forelimb movement representations

3.2.1 Methods

3.2.1.1 Ethical considerations

All procedures involving animal use in this study strictly adhered to the guidelines of the Canadian Council on Animal care and were approved by the Institutional Animal Care Committee of the University of Calgary. All efforts were made adhere to the principles of reduction, refinement, and replacement in experimental design (Russell & Burch, 1959), with every attempt made to limit the number of subjects and minimize animal suffering.

3.2.1.2 Rats

Subjects were 25 adult, male Long-Evans rats weighing 278-414 g at the time of electrophysiological mapping. All rats were obtained from the University of Calgary Breeding Colony and housed individually in clear plastic cages in a colony room maintained on a 12h light/dark cycle (lights on at 07:15) at 21°C. Mapping

experimentation was conducted during the light phase. Animals were provided free access to food (Lab Diet #5001, PMI Feeds Inc., St. Louis, MO) and water throughout the duration of their housing save for an overnight food restriction prior to electrophysiological mapping. Additionally, free access to a liquid diet (AIN-76, Bioserve, Frenchtown, NJ) was provided for three days following surgery. All animals were able to resume a solid diet by this time point.

3.2.1.3 Experimental groups and procedures

Rats were randomly and assigned into bilateral lesion (n=9), left hemisphere unilateral lesion (n = 8) and sham lesion vehicle control groups (n=8). Rats were given 5 days to acclimatize to the housing environment prior to 6-OHDA infusion surgery. Two weeks following surgery, rats underwent high-resolution intracortical microstimulation (ICMS) of the left sensorimotor cortex, described below, to derive forelimb movement representations.

3.2.1.4 Lesion protocol

Rats were anaesthetized with isoflurane (4% induction, 1.5% maintenance; VIP-3000 Vaporizer, Matrix, Orchard Park, NY) and placed in a stereotaxic instrument (Kopf, Tujunga, CA) with the incisor bar set to skull flat. Subjects were monitored continuously throughout the surgery and levels adjusted as needed to maintain a surgical level of anaesthesia. The local anaesthetic lidocaine 2% was administered subcutaneously at the incision sites.

The lesion protocol was adapted from Ben et al. (1999). Lesions were made by double bilateral infusions of 8 μg of 6-OHDA hydrochloride (Sigma, Oakville, ON) in 2 μl of physiological saline containing 1 % ascorbic acid per site (4 sites in total) at a rate of 0.5 μl/min via 26-gauge microsyringe (Hamilton, Reno, NV) afixed to a manual microdrive manipulator (Kopf, Tujunga, CA). The microsyringe was then left in place for an additional minute to aid infusate diffusion. Coordinates relative to bregma were: AP +1.7, ML ± 2.8, DV -5.6 mm & AP – 0.92, ML ± 4.0, DV -5.5 mm. Control animals received infusions of 2 μl of physiological saline containing 1% ascorbic acid at the same coordinates. Unilateral lesion animals received 6-OHDA infusions in the left striatal sites, and ascorbic acid stabilized saline vehicle in the right. Following infusions, 2% xylocaine jelly was applied around the incision and the scalp was sutured.

3.2.1.5 Electrophysiological mapping

Standard intracortical microstimulation (ICMS) techniques were used to generate detailed threshold maps of forelimb regions of the motor cortex (Kleim et al., 1998; Nudo et al., 1990; Teskey et al., 2002). Rats were anaesthetized with ketamine hydrochloride (100 mg/kg i.p.) and xylazine (5 mg/kg i.p.). Supplemental injections of either ketamine (25 mg/kg) or a mixture of ketamine (17 mg/kg) and xylazine (2 mg/kg) were given i.p. as required throughout surgery to maintain a constant level of anaesthesia as determined by monitoring vibrissae whisking, breathing rate, and foot and tail reflex in response to a gentle pinch.

A 7 x 5 mm craniotomy was performed over the left motor cortex. The window roughly extended between 4 mm anterior to and 3 mm posterior to bregma and from

midline to 5 mm lateral of midline. A small puncture was also made in the cisterna magna to reduce cortical edema. Dura was removed and silicone fluid (Factor II, Inc. Lakeside, AZ) heated to body temperature was used to cover the cortical surface. A 32x image of the exposed portion of the brain was captured using digital camera (Canon Canada Inc., Mississauga, ON) coupled to a Stemi 2000-C stereomicroscope (Carl Zeiss, Thornwood, NY), and was displayed on a personal computer. A grid of 500 μm squares was then overlaid on the digital image using Canvas imaging software (version 9.0.1, ACD systems Inc., Miami, FL). Penetrations were performed at the intersections of the grid lines and in the center of each square to give an interpenetration distance of 353 μm, except when located over a blood vessel in which case a penetration was not performed.

Microelectrodes were made from borosilicate glass capillary tubes (World Precision Instruments, Sarasota, FL) using a micropipette puller (Kopf, Tujunga, CA), filled with 3.5 M NaCl, and bevelled at 30 degrees to yield a 3 μm tip with impedance values ranging between 1.0 and 1.5 MΩ. Electrodes were guided into the neocortex to a depth of 1,550 μm by a microdrive (Narishige, Tokyo). This depth from cortical surface corresponded to the soma region of neocortical layer V pyramidal neurons. Electrical stimulation was delivered via an isolated stimulator (A-M Systems, Carlsborg, WA). A stimulation train consisted of 13 monophasic cathodal pulses, each 200 μs in duration, delivered at a frequency of 333 Hz, with each train repeating every second.

Animals were maintained in a prone position, with the right forelimb supported by placing one finger below the elbow joint and elevating the forelimb to allow visual inspection of all possible forelimb movements. To determine a movement threshold, current intensity started at 0 μA and was rapidly increased, until a movement was elicited

or to maximum of 60 μA, and then decreased until the movement was no longer present. Any penetration site that failed to elicit a movement up to the maximum intensity was considered non-responsive. A maximum of 10 trains of pulses were delivered to any given penetration site. The border of the forelimb motor map was first defined consisting of either non-forelimb movements (neck, jaw, vibrissae, trunk, tail, hindlimb) or non-responsive points in a systematic fashion. Once the border of the maps was defined, the central, forelimb regions (Neafsey et al., 1986; Neafsey and Sievert, 1982) were then determined. Forelimb movements were classified as either distal (wrist/digit) or proximal (elbow/shoulder). This procedure was used to minimize the likelihood of the microstimulation session affecting the map boundaries (Nudo et al., 1990). Throughout the surgery, anaesthetic levels were additionally monitored by verifying the thresholds for previously defined positive-response sites. Canvas imaging software was used to calculate the aerial extent of the proximal and distal representations of the caudal (CFA) and rostral (RFA) forelimb regions. Mean stimulation thresholds for each movement category and forelimb region were also calculated.

3.2.1.7 TH+ immunocytochemistry

Immediately following electrophysiological mapping, rats were deeply anaesthetized with sodium pentobarbital and perfused through the heart with cold 0.1 M phosphate buffer solution (PBS) followed with cold 4% paraformaldehyde in PBS. Brains were then extracted and postfixed in 4% paraformaldehyde in PBS. The tissue was then cryoprotected in 20% sucrose for two days. The tissue was cut in 40μ sections on a sliding microtome and collected into 0.1 M phosphate buffer containing 0.02% sodium

azide. Free-floating sections were then stained for tyrosine hydroxylase (TH) immunocytochemistry. Sections were rinsed in 0.5% hydrogen peroxide in PBS-Triton X (0.3 % Triton X-100 in 0.1M PBS) for 15 minutes to inactivate endogenous peroxidases. Three subsequent 5 minute PBS-Triton X rinses were conducted, the tissue blocked in 1% normal goat serum in PBS-Triton X for 90 minutes, and then incubated in a anti-TH rabbit primary antibody (Chemicon, diluted 1:8000) for 48 hours at 4°C. Sections were again rinsed in 3 5-minute PBS-Triton X series, incubated in biotinylated goat anti-rabbit IgG for 90 minutes, rinsed in 3 5-minute PBS-Triton X series, placed in ABC complex (Vector, Burlingame, CA) for 60 minutes, and reacted in solution containing 24 ml tris buffer, 12.5 mg 3,3'diaminobenzidine tetrahydrochloride (DAB), 60 µl 8 % NiCl, and 80 µl of 30% H_2O_2. The DAB reaction was then quenched in a rapid series of PBS rinses and sections mounted on gelatine-coated slides which were air dried, dehydrated in an alcohol series (70, 95, 100% EtOH) and cover slipped with permount.

3.2.1.8 SNc TH+ cell counts

Mesencephalic sections were viewed with an Olympus BX51 microscope using a QImaging QICAM 1394 camera at 40x and captured using ImagePro software (Media Cybernetics). Quantification of SNc dopaminergic cell loss was adapted from Metz et al. (2004). Three separate sections through the mesencephalon between 4.8 and 5.8 mm posterior from bregma were used for analysis for each rat. Care was taken to utilize sections in which a clear boundary between the SNc and ventral tegmental area was formed by the medial terminal nucleus. Where this was not possible, SNc boundaries were established according to the stereotaxic atlas of Paxinos and Watson (1986). TH+

SNc cell counts from each of the three sections for each rat, performed manually with the aid of the cell counter plugin to ImageJ (NIH), were separately averaged for each hemisphere and used for statistical analysis. TH+ neurons were identified and defined as densely stained cell bodies visible on the sections.

3.2.1.9 Statistical analyses

Statistical analyses were conducted using SPSS 16.0 (SPSS Inc., Chicago, IL) Separate one-way analysis of variance (ANOVA) tests with Tukey HSD post hocs were used for assess group differences in forelimb map expression measures, amounts of anaesthetics delivered during electrophysiological mapping, animal weights at lesion and mapping surgeries, and TH+ cell counts in the left and right hemispheres. A change in map expression was considered as changes in total map size, organization, or movement thresholds. Map size was compared using the total forelimb map area between groups. The percent distribution of forelimb movements (proximal and distal) and regions (CFA and RFA) comprising total forelimb map area assessed changes in map organization. Threshold analyses were performed to assess group differences in the minimum current intensity required to elicit forelimb movements during ICMS. As map expression has been shown to be dependent upon depth of ketamine-xylazine anaesthesia (Tandon et al., 2008), group differences in anaesthetic levels were assessed by calculating the total amount of each drug given as a ratio of each animal's weight and the total duration of the ICMS surgery. An *a priori* significance level of .05 was used for all analyses. All data are presented as mean ± SEM.

3.2.2 Results

3.2.2.1 Histology

Immunoreactivity staining indicated that striatal 6-OHDA infusion resulted in a moderate loss of TH+ cell bodies in the substantia nigra pars compacta (see Figure 3-1). There were significant main lesion effects in the left ($F_{2,21}$ = 58.515, P < .001) and right ($F_{2,21}$ = 55.966, P < .001) hemispheres between groups. Post hoc analyses revealed significant (both P < .001) reductions in mean total TH+ SNc cell counts in bilateral lesion rats in both the left (75.3 ± 8.8) and right (70.1 ± 6.1) hemispheres compared to sham lesion controls (179.4 ± 2.3 & 179.8 ± 3.1, respectively). Similarly, unilateral lesions of the left striatum resulted in significantly (P < .001) fewer residual TH+ cell bodies in the left SNc (64.5 ± 9.3) compared to sham lesion controls (179.4 ± 2.3) and the unlesioned right SNc (165.0 ± 12.0). Mean total SNc TH+ cell counts in the left (lesioned) hemisphere in unilateral lesion rats did not differ from the those in the left hemisphere of the bilateral lesion group (P = .597). No significant differences (P = .443) in mean total TH+ cell counts were found in the right (non-lesioned) hemisphere in unilateral lesion rats (165.0 ± 11.9) compared to sham lesion controls (179.8 ± 3.1).

3.2.2.2 ICMS anaesthesia levels and body weights

No significant differences in the amounts of ketamine ($F_{2,22}$ = 2.522, P = 0.103) or xylazine ($F_{2,22}$ = 0.212, P = 0.811), as a function of body weight and duration of surgery, was observed between groups. Amounts of ketamine were 1.331 ± 0.059 mg/kg/min for bilateral lesion, 1.529 ± 0.086 for unilateral lesion and 1.549 ± 0.088 for

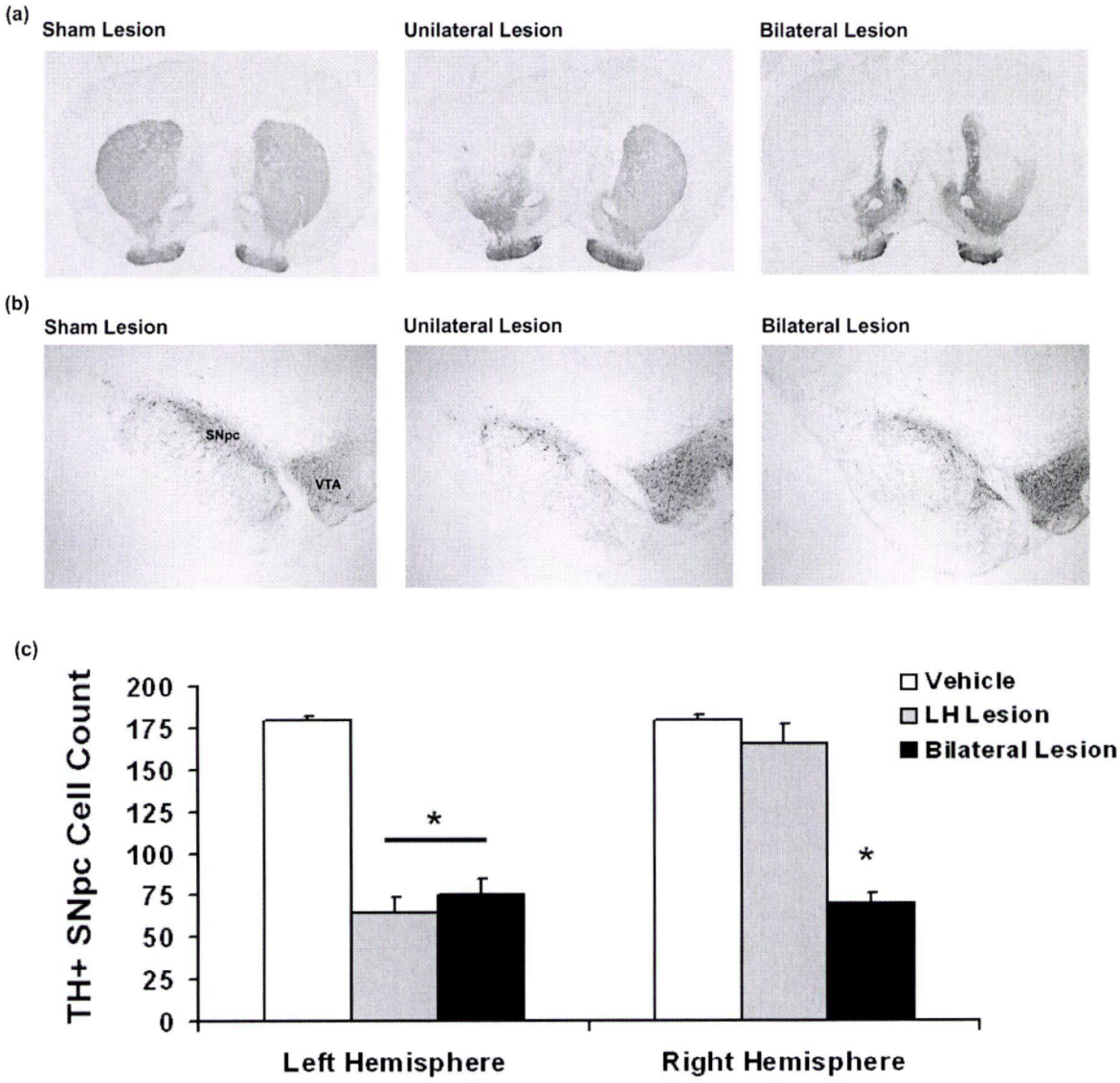
sham lesion groups. Amounts of xylazine were 0.062 ± 0.004 mg/kg/min for bilateral lesion, 0.065 ± 0.004 for unilateral lesion and 0.066 ± 0.005 for sham lesion groups. Similarly, no significant differences ($F_{2,22} = 2.429$, $P = 0.111$) in weight at the time of 6-OHDA infusion was observed between bilateral (315 ± 4.3 g), unilateral (318 ± 11.7), or sham (340 ± 9.5) lesion groups.

3.2.2.3 Neocortical movement representations

A significant main effect was observed in total area of the left neocortex able to elicit forelimb movements under ICMS stimulation ($F_{2,22} = 14.875$, $P < .001$; Figure 3-2). Post hoc analyses revealed that rats in the bilateral lesion group exhibited a significantly smaller (2.250 ± 0.328 mm^2) total map area than both left hemisphere unilateral (3.834 ± 0.219, $P = .002$) and sham (4.345 ± 0.292, $P < .001$) lesion groups. Total map area did not differ between unilateral and sham lesion groups ($P = .449$). A significant main effect was also observed for size of the caudal forelimb area ($F_{2,22} = 14.969$, $P < .001$). Bilateral lesion animals exhibited significantly smaller (1.657 ± 0.292 mm^2) caudal forelimb areas than both unilateral (2.955 ± 0.166, $P = .006$) and sham (3.668 ± 0.311, $P < .001$) lesion groups. Caudal forelimb map area did not differ between unilateral and sham lesion groups ($P = .177$).

Figure 3-1: Effects of intrastriatal 6-hydroxydopamine (6-OHDA) lesions on the density of tyrosine hydroxylase immunoreactive (TH+) staining in the striatum and the number of TH+ cell bodies in the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA). (a) Photomicrographs of coronal sections through the striatum of rats with sham, unilateral left hemisphere and bilateral 6-OHDA lesions. Left hemisphere is shown on the left. Original Magnification: 3.8x. (b) Photomicrographs of coronal sections through the left mesencephalon. A moderate reduction in SNc TH+ cell bodies is seen in lesion conditions, with a sparing of VTA TH+ immunoreactivity. Original magnification: 40x. (c) Quantification of mean total SNc TH+ cell counts in the SNc. * $P < .05$.

Figure 3-1: TH+ immunoreactivity in the striatum and substantia nigra


(a)
Sham Lesion
Unilateral Lesion
Bilateral Lesion
(b)
Sham Lesion
Unilateral Lesion
Bilateral Lesion
SNpc
VTA
(c)
TH+ SNpc Cell Count
200
175
150
125
100
75
50
25
0
Vehicle
LH Lesion
Bilateral Lesion
*
*
Left Hemisphere
Right Hemisphere

No significant differences were observed in the proportion of either caudal or rostral forelimb areas in terms of total forelimb map area ($F_{2,22} = 0.854$, $P = .439$; Figure 3-3).

There was a significant main effect in the map area eliciting distal movements ($F_{2,22} = 7.856$, $P < .001$). Bilateral lesion animals (2.208 ± 0.329 mm^2) had significantly smaller distal representations compared to both unilateral (3.724 ± 0.329 mm^2; $P = .002$) and sham (3.970± 0.329 mm^2; $P < .001$) lesion groups. No differences in distal movement area were observed between unilateral and sham lesion groups ($P = .538$). A significant main effect in the map area eliciting proximal movements was also observed ($F_{2,22} = 7.856$, $P = .004$). Sham lesion animals (0.375 ± 0.081 mm^2) had significantly larger proximal representations compared to both bilateral (0.043 ± 0.030 mm^2; $P = .004$) and unilateral (0.111 ± 0.077 mm^2; $P = .025$) lesion groups. No significant differences in proximal movement area were observed between bilateral and unilateral lesion groups ($P = .737$).

When the proportion of distal movement areas to total map area was assessed, a significant main effect was observed ($F_{2,22} = 5.537$, $P = .011$; Figure 3-4). The percent of total forelimb area eliciting distal movements in sham lesion animals (91.43 ± 1.83%) was significantly less compared to both bilateral (98.08 ± 1.31, $P = .016$) and unilateral (97.53 ± 1.54, $P = .032$) lesion groups. No differences in the percent of total forelimb area eliciting distal movements between bilateral and unilateral lesion groups ($P = .966$). Conversely, a significant main effect in the proportion of proximal movement areas to total map area was observed ($F_{2,22} = 5.537$, $P = .011$ Figure 3-4). The percent of total forelimb area eliciting proximal movements in sham lesion animals (8.57 ± 1.83%) was significantly greater compared to both bilateral (1.92 ± 1.31, $P = .016$) and unilateral

(2.47 ± 1.54, P = .032) lesion groups. No differences were observed in the percent of total forelimb area eliciting distal movements between bilateral and unilateral lesion groups (P = .966).

Threshold current intensities to elicit forelimb movements were 28.0 ± 1.3 μA for bilateral lesion animals, 28.7 ± 1.2 μA for unilateral lesion animals and 24.0 ± 2.0 μA and did not significantly differ between groups ($F_{2,22}$ = 2.574, P = .099).

Figure 3-2: Neocortical forelimb movement representations. (a) Coded forelimb motor representations of the left neocortex derived by intracortical microstimulation (ICMS) for the threshold maps of sham (top), unilateral left hemisphere (middle), and bilateral (bottom) intrastriatal 6-hydroxydopamine (6-OHDA) lesion groups. Map boundaries are defined as electrode penetration stimulations ≤ 60 μA that failed to elicit movement or elicited non-forelimb movements. A division between caudal (CFA) and rostral (RFA) map regions can be seen. Solid black vertical line indicates location of bregma in the coronal plane. (d) Mean total cortical forelimb map areas and associated standard error bars (mm^2). Bilateral 6-OHDA lesions reduced total map area. Asterisks indicate significance: * $P < .05$.

Figure 3-2: Neocortical forelimb movement representations with 6-OHDA lesions

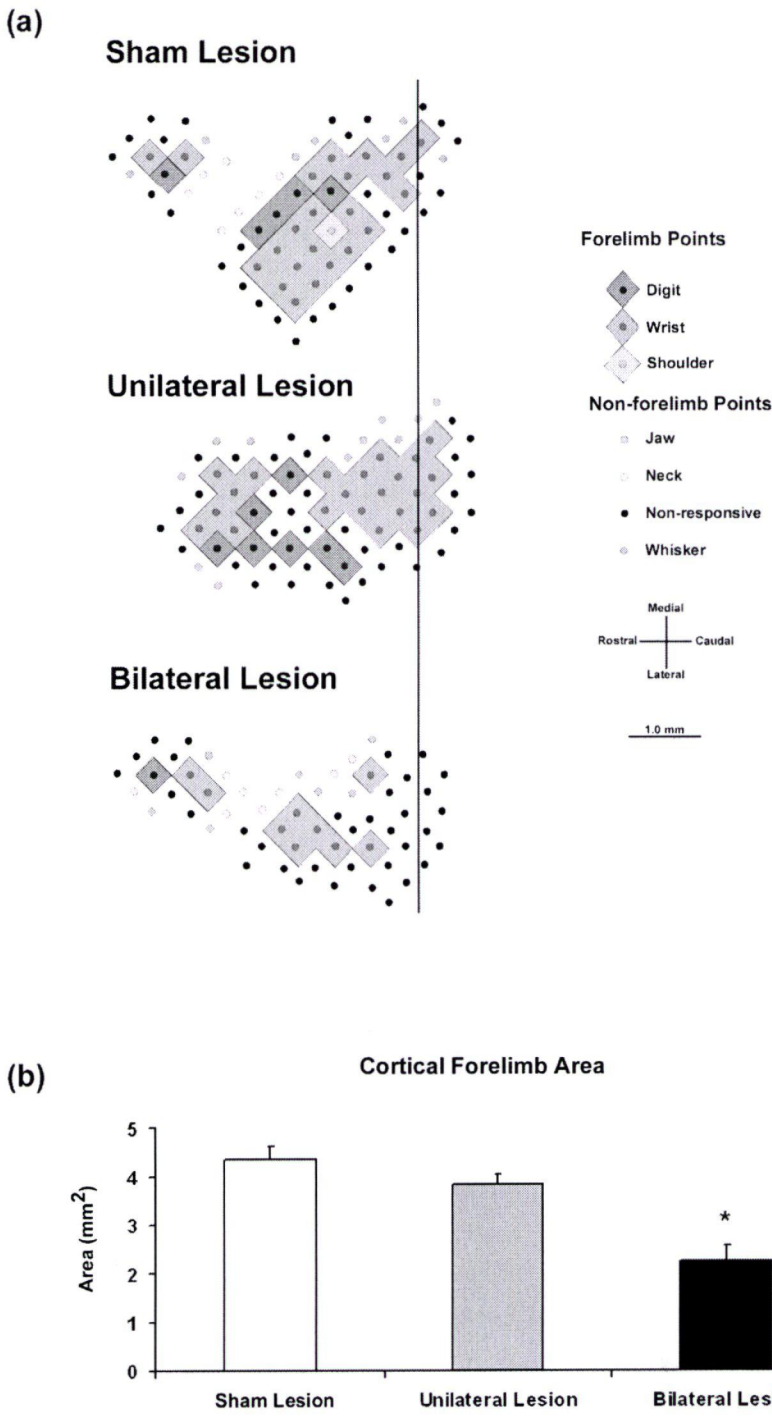


Figure 3-3: Forelimb motor map region distribution. Percent of caudal (CFA) and rostral (RFA) map areas in total cortical forelimb area (%) for sham, unilateral left hemisphere, and bilateral 6-hydroxydopamine (6-OHDA) lesion groups. No differences were observed between groups ($P > .05$).

Figure 3-3: Forelimb area distribution with 6-OHDA lesions

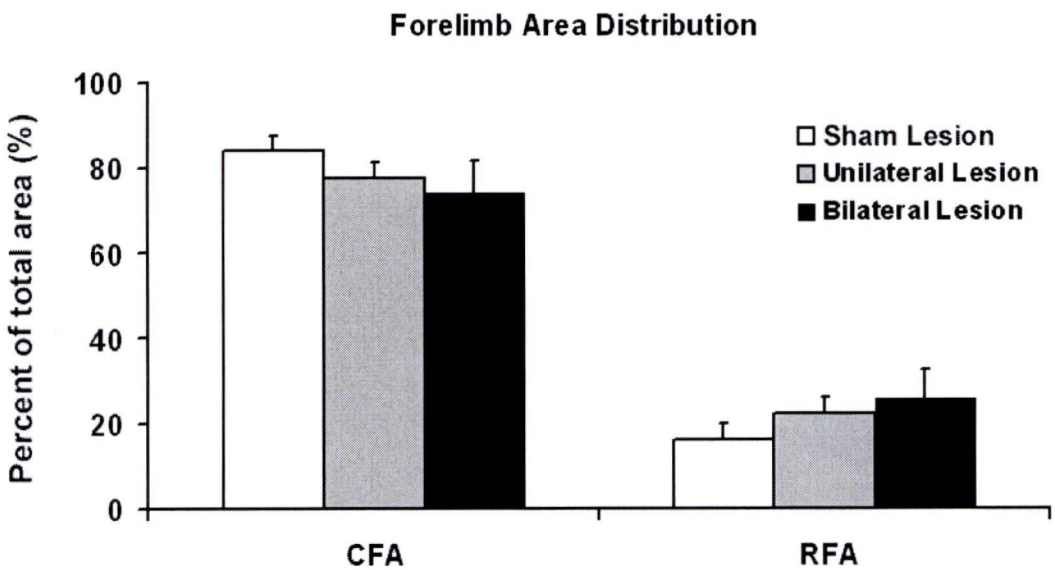
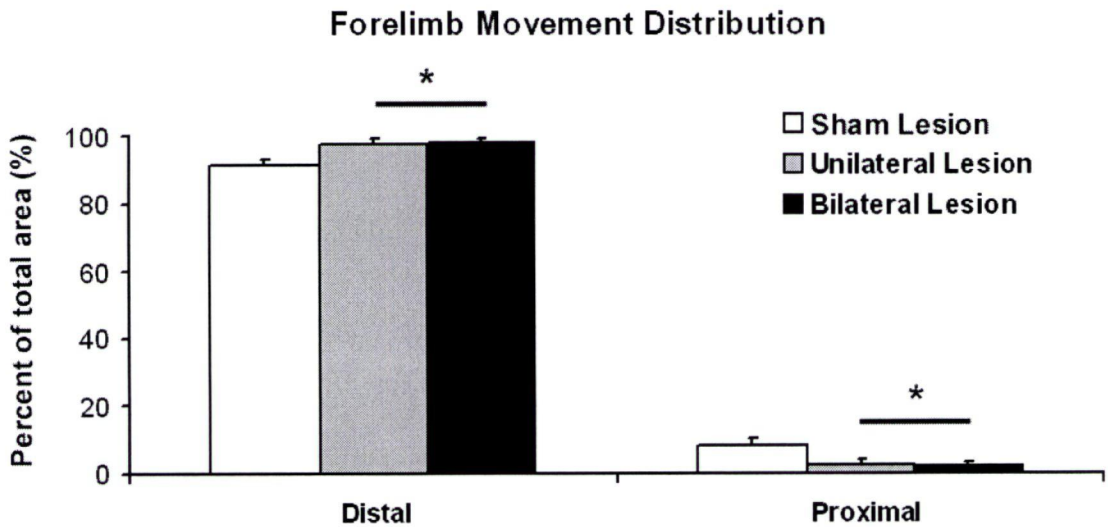


Figure 3-4: Forelimb motor map movement distribution. Percent distal and proximal forelimb representation in total cortical map area (%). Bilateral and unilateral left hemisphere 6-hydroxydopamine (6-OHDA) lesion groups exhibited a significantly greater proportion of distal movements and smaller proportion of proximal movements compared to sham lesion animals ($P < .05$).

Figure 3-4: Forelimb movement distribution with 6-OHDA lesions



3.3 Experiment II:

Acute systemic haloperidol on expression of neocortical forelimb movement representations

3.3.1 Methods

3.3.1.1 Rats

Subjects were 8 adult, male Long-Evans rats weighing 311– 417 g at the time of electrophysiological mapping housed in the same manner as in experiment I.

3.3.1.2 Experimental groups and treatments

Subjects were divided into two groups: those receiving i.p injections of either 5 mg/kg Haloperidol (n = 4) or vehicle (n=4) fifteen minutes prior to commencement of electrode penetrations during electrophysiological mapping.

3.3.1.3 Drugs

Haloperidol (Sigma-Aldrich, St. Louis, MO) was dissolved in a 10% acetic acid solution, the pH adjusted to 6 with NaOH (Håkansson et al., 2005), and brought to a final concentration of 0.5 mg/ml with 0.9 % saline.

3.3.1.4 Electrophysiological mapping

ICMS protocols were identical to those in Experiment I.

3.3.1.5 Statistical analyses

Statistical analysis were conducted using the SPSS 16.0 (SPSS Inc., Chicago, IL) Two-tailed Student *t*-tests were to assess group differences in forelimb map expression measures, amounts of anaesthetics delivered during electrophysiological mapping, and animal weights at mapping. All of these measures were assessed in the same fashion as in Experiment I. An *a priori* significance level of .05 was used for all analyses. All data are presented as mean ± SEM.

3.3.2 Results

3.3.2.1 ICMS anaesthesia levels and body weights

No significant differences in the amounts of ketamine ($t_6 = 1.125$, $P = .304$) or xylazine ($t_6 = 1.643$, $P = .304$), as a function of body weight and duration or surgery, administered between groups was observed. Amounts of ketamine were 1.659 ± 0.112 mg/kg/min for vehicle control and 1.499 ± 0.088 for haloperidol treated animals. Amounts of xylazine were 0.072 ± 0.002 mg/kg/min for vehicle control and 0.066 ± 0.003 for haloperidol treated animals. Furthermore, no significant differences in weight between vehicle control (382 ± 18 g) and haloperidol treated rats (345 ± 13) was observed between groups at the time of electrophysiological mapping ($t_6 = 1.729$, $P = .135$).

3.3.2.2 Neocortical movement representations

The cortical area able to elicit forelimb movements significantly differed between groups (t_6 = 4.131, P = .006). Total stimulation-induced forelimb area in haloperidol treated rats (4.404 ± 0.480 mm^2) was 50% that of vehicle controls (2.067 ± 0.299; Figure 3-5). The smaller forelimb movement representation in the haloperidol group was due to significant reduction in both caudal (t_6 = 3.339, P = .016) and rostral (t_6 = 2.557, P = .043) forelimb area compared to vehicle controls. Caudal forelimb representations were 1.750 ± 0.261 mm^2 for haloperidol treated rats and 3.741 ± 0.536 for vehicle controls. Rostral forelimb representations were 0.317 ± 0.122 mm^2 for haloperidol treated rats and 0.662 ± 0.059 for vehicle controls. The smaller forelimb movement representation in the haloperidol group was due to proportional reduction in both caudal and rostral forelimb areas as their distribution in terms of the total did not differ between groups (t_6 = 0.115, P = .882; Figure 3-6). The caudal forelimb area comprised 85.01 ± 6.39% of total forelimb area in haloperidol treated animals and 83.99 ± 2.99 in vehicle controls. Similarly, the rostral forelimb region comprised 15.10 ± 6.36 % of the total forelimb area in haloperidol treated animals and 16.01 ± 2.99 in vehicle controls (See Figure 3-5).

When comparing differences in the forelimb map area subserving distal and proximal movements and taking into account the total size of the map, haloperidol induced map area reduction was not associated with a reorganization of forelimb movement representations. There was a significant (t_6 = 3.374, P = .015) reduction in total map area eliciting distal movements in haloperidol treated animals (2.035 ± 0.302 mm^2) compared to vehicle controls (3.610 ± 0.356), but no difference (t_6 = 1.448 , P = .198) in map area eliciting proximal movements between haloperidol (0.032 ± 0.032) and

vehicle (0.794 ± 0.525) treatments. When these areas were assessed in terms of their magnitude to the total forelimb map area (Figure 3-6), the assumption of homogeneity of variance between groups was shown to be violated by Levene's test ($P < .05$) and Welch's *t*-tests were used to compare the percentage of total map area eliciting distal and proximal forelimb movements. Distal movement representations accounted for 98.42 ± 1.58 % of the total forelimb map area in haloperidol animals and did not differ ($t_{3.172}$ = 1.517, $P = .222$) from vehicle controls (84.10 ± 9.31). Similarly, proximal movement representation accounted for 1.58 ± 1.58 % of the total forelimb map area in haloperidol animals and did not differ ($t_{3.172} = 1.517$, $P = .220$) from vehicle controls (15.900 ± 9.310).

Threshold current intensities to elicit forelimb movements were (28.6 ± 2.0 μA) for haloperidol treated animals and did not differ ($t_6 = 1.701$, $P = .140$) from those of vehicle controls (23.7± 2.1).

Figure 3-5: Threshold forelimb motor representations derived by intracortical microstimulation. Color-coded representation of the total left neocortical forelimb area for the threshold map of a (a) vehicle control and (b) haloperidol-treated rat. Map boundaries are defined as electrode penetration stimulations ≤ 60 μA that failed to elicit movement or elicited non-forelimb movements. A clear division between caudal (CFA) and rostral (RFA) map area can be seen with these boundaries. Solid black line indicates location of bregma in the coronal plane. (d) Mean total forelimb map areas (mm^2) and associated standard error bars. Haloperidol treatment significantly reduced total map area compared to vehicle. Asterisks indicate significance: * $P < .05$

Figure 3-5: Neocortical forelimb movement representations under haloperidol

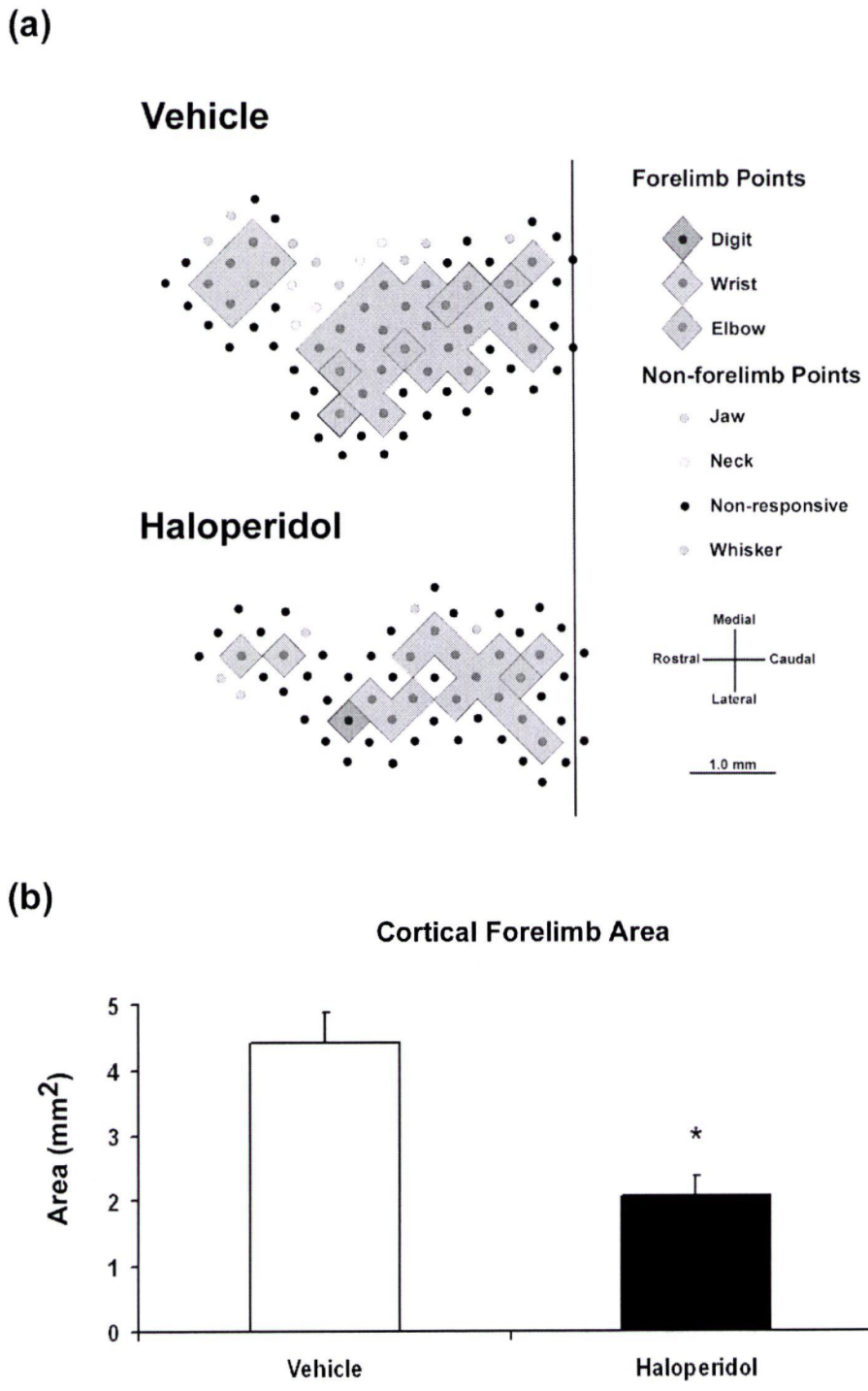


Figure 3-6: Forelimb motor map region distribution. Percent of caudal (CFA) and rostral (RFA) map areas in total cortical forelimb area (%). No differences were observed between vehicle and haloperidol treatment groups ($P > .05$).

Figure 3-6: Forelimb area distribution under haloperidol

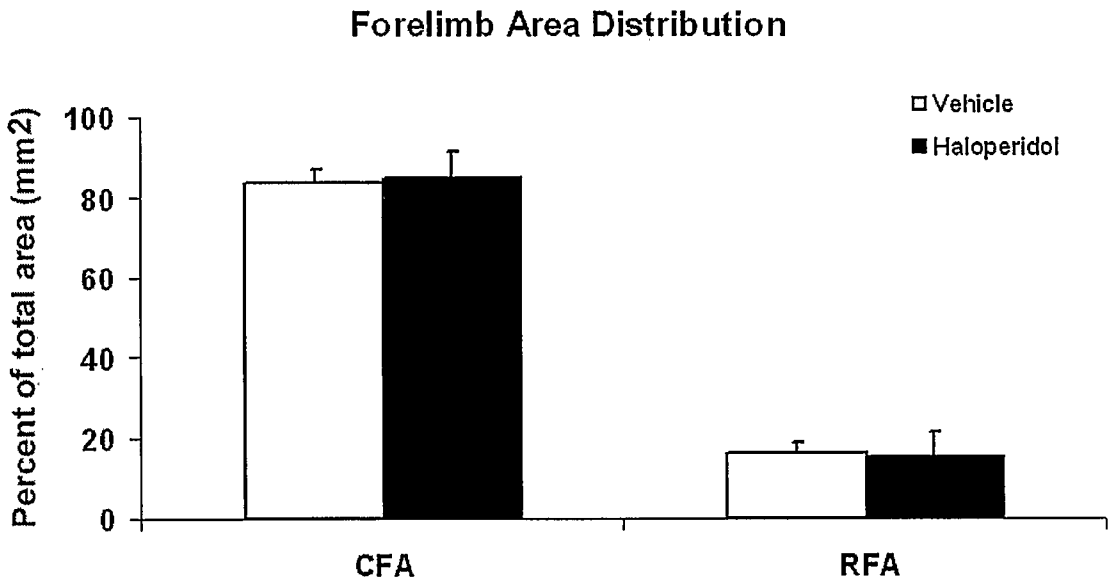
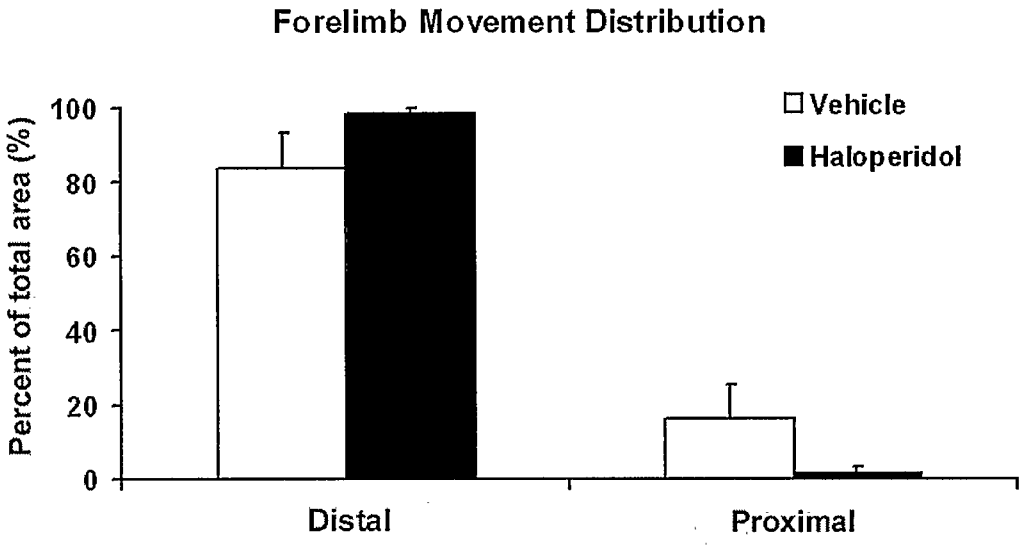


Figure 3-7: Forelimb motor map movement distribution. Percent distal and proximal forelimb representation in total cortical map area (%). No differences were observed between vehicle and haloperidol treatment groups ($P > .05$).

Figure 3-7: Forelimb movement distribution under haloperidol



3.4 Discussion

The present results have shown that bilateral degeneration of nigrostriatal circuitry induced by intrastriatal application of 6-OHDA significantly reduces forelimb motor map expression in the rat. Changes in map expression, as delineated by ICMS, presented as a reduction in total forelimb map area and a map reorganization characterized by an increase in the size of distal-to-proximal forelimb movement representation compared to sham lesion controls. The observed map changes were not dependent on anaesthetic level differences (Tandon et al., 2008) between groups. Furthermore any changes in map expression as a result of cortical damage from the infusion tract were accounted for in sham lesion controls. ICMS stimulation parameters in the present study gave rise to a similar map expression in the control condition as reported previously (Teskey et al., 2002; Metz et al., 2004; Teskey and Monfils, 2004; Teskey et al., 2007; Ozen et al., 2008). Additionally, no differences in movement thresholds were observed, further supporting dissociation between map area and movement thresholds (Teskey et al., 2002).

Map expression is sensitive to both local injury to the sensorimotor cortex (Nudo and Milliken, 1996) and damage of the corticospinal tract (Piecharka et al., 1995). Presented here is evidence that map integrity is impacted by damage to nigrostriatal projections. An interesting interpretation of this finding is that map expression may be modulated by basal ganglia-thalamocortical afferents to the sensorimotor cortex (Rouiller et al., 1993) to the extent that bilateral and unilateral, ipsilateral-to-map nigrostriatal degeneration was associated with map alterations. The motor map hypotrophy was very

dramatic for bilateral striatal lesions. Given that maximal stimulation intensity in many loci was unable to elicit movements in these rats, map hypotrophy is likely reflective of reduced excitability in motor neurons and/or enhanced intracortical inhibition. These results support the basal ganglia-thalamocortical circuit model predictions of PD which proposes a hypoactivity of the frontal neocortex due to a hyperactivity of basal ganglia output circuitry resulting from nigrostriatal degeneration (Alexander et al., 1990; DeLong and Wichmann, 1993; Wichmann and DeLong, 2003; Braak and Del Tridici, 2008). Furthermore, the present results are consistent with previous findings of reduced cortical activation in 6-OHDA lesion rats as indicated by reduced metabolic activity (Orieux et al., 2002) and immediate early gene expression (Steiner and Kitai, 2000) in frontal cortical areas.

Unilateral 6-OHDA infusions did not result in a change in contralateral forelimb map area, replicating findings from unilateral 6-OHDA lesions of the medial forebrain bundle (Metz et al., 2004). Preservation of map area was observed in ipsilateral unilateral lesion rats that had 58 to 64% loss of TH+ SNc cells. Similar to bilateral 6-OHDA infusions, unilateral lesions also resulted in a significant increase in the proportion of total map area eliciting proximal movements compared to sham lesion controls. Subtle map reorganization was also observed by Metz et al. (2004) in that the distal representation in the caudal forelimb area of the lesioned rats was larger than that of sham lesion controls, while the distal representation in the rostral forelimb area of lesioned rats was smaller than that of sham lesion controls. It is known that the effects of unilateral nigrostriatal degeneration in rats are not restricted to the lesioned hemisphere. Changes in basal ganglia output (Breit et al., 2008) and a marked hypertrophy of output

layer pyramidal cell dendritic arbours in the forelimb sensorimotor cortex (Miklyaeva, et al., 2007) are observed in the non-lesioned hemisphere. Perhaps neuroplastic changes in the non-lesioned hemisphere are able to partially compensate for lesion effects in the injured hemisphere. It seems unlikely that residual nigrostriatal dopaminergic fibers are involved as Metz et al. (2004) observed near complete nigrostriatal cell loss in the lesion hemisphere, and unilateral 6-OHDA MFB lesions result is near complete loss of DA innervation in the striatum that does not improve significantly with time (Deumens et al., 2002). Furthermore, bilateral lesion animals in the present study exhibited significantly smaller forelimb maps with moderate preservation of residual nigrostriatal fibers. Indirect compensatory mechanisms mediated by subcortical structure such as the pedunculopontine nucleus (PPN; Lavoie and Parent, 1994; Charara et al., 1996; Kitai, et al., 1999; Breit et al., 2008) or the contralateral sensorimotor cortex (Miklyaeva et al., 2007) seem more likely and may explain subtle lesion effects on map organization without an effect on map size. The classical basal ganglia-thalamocortical circuit model predominately relies on a unilateral representation, with little attention given to interhemispheric projections. It has been widely recognized that the effects of unilateral nigrostriatal degeneration are not restricted to the lesioned hemisphere (Vergara-Aragon et al., 2003; Miklyaeva et al., 2007; Woodlee et al., 2008), however the functional interactions between injured and intact hemispheres are not well understood. Results from the present study suggest that the contralateral-to-lesion hemisphere in hemiparkinsonian rats may be able to partially compensate for sensorimotor pathology from nigrostriatal degeneration by preventing a reduction in the ipsilateral-to-lesion forelimb map area.

The map expression changes associated with intrastriatal 6-OHDA lesions are presumed to be a consequent of nigrostriatal degeneration, however DA-depletion produces motor abnormalities which can influence map expression (Kleim et al., 2003; Plautz et al., 2003). In an attempt to mitigate the influence of lesion-induced behavioural deficits on map expression, all animals were housed individually in a deprived condition between lesion and electrophysiological mapping surgeries. It is known that increased limb use in the absence of motor skill acquisition does not result in a net change in either movement representation topography in non-human primates (Nudo et al., 1997) or synapse number in the sensorimotor cortex of the rat (Kleim et al., 1996), but the effects of decreased limb use on map organization have not been investigated. Behavioural deprivation has been shown to decrease the size distal-to-proximal movement representations relative to an increase in distal-to-proximal movement representations from skilled motor enrichment (Kleim et al., 1998; Remple et al., 1999). In the present study both bilateral and unilateral lesion rats exhibited an increase in ratio of distal-to-proximal representations compared to sham lesion animals. If motor impairment could be considered an extreme case of deprivation, the opposite finding would have been expected. On the other hand, mild sensorimotor deprivation in a single housing environment similar to the one used in this study for up to four months did not induce a significant reduction in the cutaneous area of the forepaw sensory map, but severe sensory deprivation in form of forced forelimb disuse for two weeks resulted in a large decrease in cutaneous forepaw representation in the casted limb (Coq and Xerri, 1999). The extent that sensorimotor impairment may have impacted map expression was not determined in this study. However, the impairments were not so severe as to prevent the

animals from feeding, as all rats were able to return to the solid diet within three days of the lesion. Furthermore, Metz et al. (2004) did not find any change in map expression in hemiparkinsonian rats from skilled motor training. Therefore, it seems unlikely that present results are influenced to any great degree by behavioural impairments.

To provide further evidence that the present results are a direct consequence of dopamine depletion, the second experiment of this study addressed whether dopaminergic antagonism alone is able to reduce forelimb map expression. Acute systemic haloperidol treatment was shown to significantly reduce the area of forelimb motor maps. This finding further supports the notion that bilateral reductions in dopaminergic tone are required for reductions in total map area. Haloperidol is a selective D2 receptor antagonist and present findings indicate that D2 antagonism alone is sufficient for exerting changes in map expression. D2 receptor antagonism in the primary motor cortex has been shown to significantly reduce forelimb motor map expression as delineated by surface electrode grid mapping (Hosp et al., 2008), and present results agree with and extend this finding by showing that acute systemic D2 antagonism results in a similar map hypotrophy as revealed by ICMS. There was a non-significant trend for increased distal-to-proximal forelimb movement representation in haloperidol treated animals compared to controls that was in agreement with results the significant results from intrastriatal 6-OHDA lesions. As there was a relatively small sample size in this experiment, an increased sample may prove a significant difference in this measure and provide further evidence that bilateral lesion map changes are a direct result of dopamine depletion.

In summary, our results are consistent with basal ganglia-thalamocortical model by demonstrating that bilateral depletion of striatal dopamine in the rat can induce a significant reduction in the size of neocortical forelimb movement representations, a functional marker frontal cortical hypoactivity. Further, it is suggested that reduced dopaminergic tone in the basal ganglia when occurring bilaterally will have more severe functional consequences on motor cortical excitability.

Chapter 4: General Discussion

Two investigations were pursued in this thesis. In the first study (chapter 2), a behavioural model was developed to assess whether auditory cue training in the rat is associated with changes in evoked neocortical field responses. Rats were trained to respond to a single auditory tone or a brief train of stimulation to the medial geniculate by initiating movement to collect a sucrose pellet reward in an open recording chamber. Results from this study indicate that rats in both tone and stimulation cue paradigms can be trained to respond to an otherwise neutral stimulus. A dissociation between behavioural learning and neuroplastic effects of cue training was observed. Task acquisition was shown to be associated with an increase in the peak height of the evoked geniculo-auditory field response in tone cue-trained rats, but no changes in evoked neocortical responses were observed in stimulation cue-trained rats. Furthermore, prior auditory tone cue training was showed to specifically improve performance under cataleptic challenge with haloperidol in a probe cueing task, but not in a general assay of catalepsy as reavealed in the hanging bar test.

The second study (chapter 3) tested PD pathophysiological theory predictions of frontal hypoactivity resulting from nigrostriatal dopamine cell loss (DeLong and Whichmann, 1993). As an index of frontal function, forelimb neocortical movement representation expression was assessed in the rat following nigrostriatal generation induced by intrastriatal infusion of 6-OHDA. Bilateral loss of dopaminergic SNc neurons was shown induce significant map hypotrophy, with unilateral cell loss being associated with subtle ipsilateral map reorganization. Acute systemic dopaminergic antagonism with

haloperidol was also shown to induce significant map hypotrophy. Results from this study support PD pathophysiological model predictions and suggest that bilateral reductions in striatal dopaminergic tone induce more severe functional consequences on motor cortex excitability.

Behavioural effects of sensorimotor cue training in the first study may share similarities to findings of movement facilitation in PD under external sensory cue guidance (Rubinstein et al., 2002; Morris and Iansek, 1996). However, there are some limitations of the current study that prevent a conclusive interpretation of the relationship between the neuroplastic effects of tone cue training and reduced akinesia under haloperidol. Tone cue training was only associated with increased performance in the probe cueing task under haloperidol and not a general reduction in akinesia as demonstrated in the hanging bar test. This finding suggests that improved probe cueing task performance may be due to a pre-training effect (Caldji and Vanderwolf, 1996) rather than a generalized cue-induced facilitation of movement. Second, post-training behavioural challenges were not conducted in stimulation cue-trained animals. The effect of prior cue training in these animals, which was not associated with neuroplastic effects, on these post training behavioural challenges is unknown. However, the second study demonstrated significant motor map hypotrophy from acute systemic haloperidol, suggesting that improved probe cueing task performance is observed despite severe disruption of frontal motor output activity. This finding could both indicate resistance of a previously learned sensorimotor skill to dopaminergic antagonism and suggest a role for extra-basal ganglia compensatory processes mediating increased performance in trained animals, as is posited in PD (Morris and Iansek, 1996). However, it should be

noted that haloperidol-induced map hypotrophy was observed using a much higher dose then used in the probe cueing task. The effect of the lower dose of haloperidol, used with the post-training behavioural challenges, on map expression is not known.

A novel finding of this thesis is that bilateral SNc dopamine cell loss may be required to induce severe disruptions in motor map integrity. A limitation of the study in chapter 2 is the unknown degree to which any lesion induced behavioural impairments may have influenced results (Kleim et al., 2003; Plautz et al., 2003).. While this influence on map expression was thought to be rather minimal based on previous studies (Coq & Xerri, 1999; Metz et al., 2004), an exhaustive behavioural assay of lesioned animals was not undertaken. Furthermore, acute dopaminergic antagonism with haloperidol also significantly reduced forelimb map expression. However, as haloperidol in was given systemically, reductions in map area may have been influenced by action at cortical dopamine receptors (Hosp et al., 2008).

There is evidence that during PD progression, an increased reliance on external sensory cues may lead to overactivity of the frontal neocortex through recruitment of extra-basal ganglia mechanisms (Morris and Iansek, 1996; Haslinger et al., 2001). It would then be of interest to determine whether prior sensorimotor cue training would exert protective effects on map expression under dopamine antagonism. Furthermore, as some complex sounds have shown to be innately behaviourally activating in cataleptic rats (Whishaw, 1993; Field et al., 2000; Clark et al., 2008), their acute effects on map expression under dopamine antagonism are of interest. Additionally, there is evidence that sensory input to thalamic cueing pathways (Hu, 2003; Mooney et al., 2004) may have the capacity to replace defective basal ganglia internal cue generation (Morris and

Iansek, 1996). Determination of whether learned or unlearned sensory cues that can trigger behaviour act through these pathways would further understanding of cued movement facilitation in PD.

In summary, findings of the present thesis support PD pathophysiological model assumptions and add to current knowledge by indicating the importance of interhemispheric interactions. Further, findings also indicate that the mechanisms involved in rehabilitation therapies focusing on sensory cue recruitment in PD may be modeled in the rat.

References

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