NEURAL TRANSPLANTATION IN THE RAT MODEL OF PARKINSON'S DISEASE

by K. Adam Baker

Submitted in partial fulfillment of the requirements for the degree of

Master of Science in Anatomy and Neurobiology / Neuroscience

at

Dalhousie University

Halifax, Nova Scotia

March, 2000

This thesis would not have been possible without the continued support and commitment of my fiancée, Sherri. Having brought Sherri to Nova Scotia, away from her family and friends, I realize that the past few years have not been easy for her, but she has stuck by me through the good times and the not so good times. I am eternally grateful for her continued devotion to my well-being and happiness and also for always being available to offer words of encouragement.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

List of figures

List of tables

ABSTRACT

Parkinson's disease (PD) is a severe neurodegenerative disorder afflicting approximately 1% of the population over 60 years of age. PD is marked by progressive sensorimotor disturbances that ultimately lead to disability and death. Currently there is no cure for PD, but the symptoms can be adequately controlled by administration of L-3,4-dihydroxyphenylalanine (L-Dopa). However, most patients develop intractable side effects and with long-term use and no longer respond to L-Dopa. The inability of L-Dopa to provide long-term benefits has stimulated the search for alternative strategies for the treatment of PD. Selective lesions or chronic stimulation of basal ganglia structures are currently being investigated. However, transplantation of DA-rich tissue to DA-depleted areas of the brain may hold the greatest promise of a cure for PD. However, before neural transplantation can be considered as a routine procedure for the treatment of PD, some crucial issues need to be addressed. Two important issues relate to the source of tissue for the treatment of PD and the establishment of the appropriate target(s) for transplantation. In this work, I have specifically addressed those two issues.

In the first study, I hypothesized that hNT neurons, derived from a human teratocarcinoma, can be used for neural transplantation in a rodent model of PD. Hemiparkinsonian rats received subsequent intrastriatal and intranigral grafts (double grafts) of 1) medium only; 2) hNT; 3) hNT-DA; or 4) lithium chloride (LiCl) pretreated hNT-DA neurons. Immunohistochemistry for the presence of tyrosine hydroxylase (TH) revealed TH-immunoreactive (THir) cells in the hNT-DA and LiCl pretreated hNT-DA groups, compared to no THir cells in the controls or animals with hNT neuronal grafts. This experiment demonstrated that hNT-DA neurons survive and differentiate into THir cells and LiCl pretreatment may enhance TH expression in hNT-DA cells. Although the number of cells expressing TH was relatively small, there was still evidence of some functional effects in animals with hNT-DA or LiCl pretreated hNT-DA neurons. This work has demonstrated for the first time the potential of hNT-DA neurons to be used in neural transplantation. In the second experiment, I hypothesized that the intranigral dopaminergic graft is important in the double graft strategy. Hemiparkinsonian rats were subsequently transplanted with: 1) double fetal nigral grafts or; 2) intrastriatal grafts alone. Nine weeks following transplantation, the animals were randomly subdivided into four equal-sized groups and received either intranigral injections of 1) vehicle or; 2) 6-hydroxydopamine (6-OHDA). Intranigral 6-OHDA injections in the double graft group resulted in a significant reversal of behavioural recovery, which was not exhibited by any of the other groups. Robust surviving THir intranigral grafts were observed in double grafted animals with subsequent vehicle injections compared to only small grafts in animals with subsequent 6-OHDA injections. This experiment demonstrates that the intranigral graft has an important role in the recovery of double grafted animals.

The results of the above two experiments may have important clinical relevance to the treatment of PD. The finding that non-fetal-derived cells (hNT neurons) can be survive and express TH *in vivo* may diminish our dependency on fetal tissue. Based on the work here and in previous studies by our laboratory that double grafts can restore DAergic reinnervation of the striatum and substantia nigra and that the nigral target is critical for behavioural recovery suggests that the double graft strategy may increase the functional efficacy of neural transplantation for PD.

LIST OF ABBREVIATIONS AND SYMBOLS

°C	degrees Celsius
6OH	intranigral 6-OHDA injection (Chapter 3)
6-OHDA	6-hydroxydopamine
µg	micrograms
µl	microliters
µm	micrometers
Ab	antibody
ABC-kit	avidin-biotin complex kit
ADL	activities of daily living
aFGF	acidic fibroblast growth factor
Am	amygdala
AM	adrenal medulla
ANOVA	analysis of variance
A/P	anteroposterior
AP-1	activator protein-1
AR	Arkansas
BDNF	brain-derived neurotrophic factor
bFGF	basic fibroblast growth factor
CA	California
CC	cingulate cortex
CNS	central nervous system

D1 receptor	dopamine receptor, type 1
D2 receptor	dopamine receptor, type 2
DA	dopamine
DAB	3,3'-diaminobenzidine
DBL	double grafted group of animals (Chapter 3)
DDC	dopa decarboxylase
DMEM	Dulbecco's modified Eagle's medium
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
D/V	dorsoventral
E	embryonic day
EC	entorhinal cortex
EPN	entopeduncular nucleus
FC	frontal cortex
FGF	fibroblast growth factor
FVM	fetal ventral mesencephalon
g	grams
GABA	γ-aminobutyric acid
GDNF	glial cell line-derived neurotrophic factor
GP	globus pallidus
GPe	external segment of the globus pallidus
GPi	internal segment of the globus pallidus

H_2O_2	hydrogen peroxide
HBr	hydrobromic acid
HPC	hippocampus
HRP	horse radish peroxidase
hNT neurons	neurons derived from a human teratocarcinoma
hNT-DA neurons	hNT neurons cultured treated for 4 weeks with RA
HRP	horseradish peroxidase
IC	inferior colliculus
IgG	immunoglobulin G
IL	Illinois
ip	intraperitoneal
ir	immunoreactive
kg	kilograms
L-Dopa	L-3,4-dihydroxyphenylalanine
LiCl	lithium chloride
M	molar
MAO_B	monoamine oxidase type B
MDF	muscle-derived factor
mg	milligrams
min	minutes
M/L	mediolateral
ml	milliliters

Moc1	anti-human NCAM monoclonal antibody
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
mRNA	messenger ribonucleic acid
n	number of subjects
NAc	nucleus accumbens
NCAM	neural cell adhesion molecule
NHS	normal horse serum
nl	nanoliters
NMDA	N-methyl-D-aspartate
NSE	neuron-specific enolase
NSS	normal swine serum
NT2	neuronal precursors derived from a human teratocarcinoma
NTN	neurturin
Nurr1	nuclear receptor-related factor 1
OB	olfactory bulb
OH	Ohio
P	postnatal day
PB	phosphate buffer
PBS	phosphate-buffered saline
PD	Parkinson's disease
PET	positron emission tomography
PKA	protein kinase A

PKC	protein kinase C
PPT	pedunculopontine tegmental nucleus
RA	retinoic acid
RF	reticular formation
SA	septal area
SD	standard deviation
sec	seconds
SHH	sonic hedgehog
SN	substantia nigra
SNc	substantia nigra pars compacta
SNr	substantia nigra pars reticulata
ST	stria terminalis
STN	subthalamic nucleus
STR	striatum (Figure 4.1) or intrastriatal grafted group (Chapter 3)
TH	tyrosine hydroxylase
VEH	intranigral vehicle injection (Chapter 3)
Vim	ventral intermediate nucleus of the thalamus
VM	ventral mesencephalon
VTA	ventral tegmental area
WA	Washington

ACKNOWLEDGEMENTS

There are so many people to acknowledge for making the past few years at Dalhousie not only rewarding but also productive. First, I would like to acknowledge the 1% of Canadians over 60 years of age who are afflicted with PD. For without them or my desire to find an effective treatment strategy for their debilitating disorder, this thesis may never have been written. Secondly, I would like to thank Dr. Ivar Mendez, not only for giving me the opportunity to pursue my Master's degree in his laboratory, but also for the strong support and supervision he has provided me. Working with Dr. Mendez has made me realize the many qualities that are required to become successful as an independent researcher. While everyone in the laboratory has their own independent research project, Dr. Mendez has fostered a feeling that each project is not to be considered trivial or not as important as another project. Each project is carefully designed to make small steps towards answering a bigger question with the ultimate goal of improving the quality of life of those afflicted with neurological disorders, such as Parkinson's disease and spinal cord injury. I am eternally grateful to Dr. Mendez for all that he has done and rest assured that the next few years as a Ph.D. student in his laboratory will be as equally rewarding.

I may be biased, but I feel that the Neural Transplantation Laboratory is staffed with one of the finest technicians. Mr. Damaso Sadi has been invaluable to many of the studies undertaken in our laboratory. Not only is Damaso knowledgeable on the day-to-day runnings of the laboratory, but he also tries to find the time to help all of the students with their projects. I not only admire Damaso for his extreme efficiency at handling tasks in the laboratory, but he is truly an inspiration to anyone contemplating having a family

in the future. Although Damaso's main priority is his family, he is able to efficiently juggle his family commitments without compromising his responsibilities in the laboratory. Within the past year, Mr. Ruperto Ulalia and Ms. Tanya Myers have joined the Neural Transplantation Laboratory team (or family, depending on your point-of-view) and are proving welcome additions to the laboratory.

During the last few years many students have come through the laboratory. The honest and open nature and willingness to assist make Mr. Karim Mukhida a special person and a good friend. Ms. Melanie Altas' and Ms. Kimberley Butler's light-heartedness and mature attitudes have made them truly a pleasure to work with and talk with. With the good times always comes times of great stress. When the stress in the laboratory is at a peak, the 'child-like' view-point or care-free attitude of Ms. Aylin Alemdar always lightens the mood. Other past and present members of the laboratory worthy of acknowledging include: Mr. Adam Hebb, an exciting roommate, Dr. Murray Hong for his guidance and Ms. Asraa Al-Mosawie for her honesty and sincerity. I would also like to acknowledge Ms. Dianne Duncan, Ms. Tanya Acorn and Ms. Pat Turner for their assistance with various tasks over the past couple of years. Everyone mentioned above has helped in some way to make the writing of this thesis possible.

I would like to acknowledge the continued support and commitment of my family and most notably my fiancée, Ms. Sherri Sutherland. Without their continued love and support, none of this may have been possible. I will always treasure them for all that they have done for me in the past and continue to do for me now.

A few years ago, it was thought that my university career might be over but the

constant support that I have received from my church family has helped me to keep pushing forward. These same people gathered together before our departure to Dalhousie and offered us money to help offset the cost of relocating to the East Coast from Southern Ontario. For this I am eternally grateful. Most of all I would like to thank God for allowing me the opportunity to accomplish what I have. I would also like to thank all of the specialists at University and Parkwood Hospitals in London, Ontario and Niagara Rehab in St. Catharines, Ontario for their help and support in my return to the academic setting, for without them I am not sure if any of this may have been possible.

Chapter 1:

Introduction

Parts of this chapter have been taken from works published in Brain Research Reviews (2000) 32: 328-339 and the Dalhousie Medical Journal (1998) 26: 25-32.

Overview and hypotheses

Parkinson's disease (PD) is a severe neurodegenerative disorder resulting from the selective loss of the dopaminergic neurons of the substantia nigra pars compacta (SNc). Degeneration of these neurons leads to a dramatic reduction in dopamine (DA) levels in the striatum, which is the main target of the DAergic SNc neurons. The mainstay of treatment for PD, involves the administration of the precursor to DA biosynthesis, L-3,4-dihydroxyphenylalanine. Although L-Dopa is initially effective in alleviating parkinsonian manifestations, the beneficial effects of L-Dopa often wear off with time and there is the development of neuropsychiatric symptoms, motor fluctuations (the 'on-off' phenomenon) and abnormal movements or dyskinesias (Olanow et al., 1996). Intractable side effects and the decreased efficacy of medical (drug) therapy for PD over the long-term has stimulated the search for surgical alternatives for the treatment of this devastating disorder. Of the surgical options currently being investigated, neural transplantation holds the greatest potential of a cure for PD. The results of clinical trials reported thus far on neural transplantation are promising but several issues need to be addressed before neural transplantation can be viewed as a treatment strategy for PD (Olanow et al., 1996; Mehta et al., 1997). In the present work, two critical issues in neural transplantation have been investigated: 1) the use of alternative sources to fetal tissue for neural transplantation of DA-rich cells; and 2) the optimal target site(s) for neural transplantation.

My work has focussed in testing the following hypotheses in an attempt to answer the questions stated above.

1) That hNT neurons, derived from a human teratocarcinoma cell line (NT2) can be used for neural transplantation in the rat model of PD.

2) Reinnervation of the substantia nigra is important in a simultaneous intrastriatal and intranigral (double graft) grafting strategy in the rat model of PD.

Parkinson's disease

In 1812, James Parkinson first described a disorder characterized by bradykinesia, gait and speech disturbances, tremor and a flexed posture, which became known as Parkinson's disease (Youdim and Riederer, 1997). Presently, PD afflicts approximately 1% of the Canadian population over the age of 60. PD results from the selective loss of the dopamine (DA)-containing neurons, most predominantly being the DAergic neurons of the SNc, that project predominantly to the striatum and are important in motor function. When >80% of the neurons of the SNc have degenerated, the cardinal symptoms of PD begin to emerge (Olanow et al., 1996).

The cause of PD is currently unknown, but an Italian-American family has recently been discovered in which their form of early-onset PD is inherited, and appears to result from mutations in the *α-synuclein* gene (Polymeropoulos et al., 1997; Papadimitriou et al., 1999). Transcription of the *α-synuclein* gene liberates a protein that has been hypothesized to integrate presynaptic signalling and be involved in membrane trafficking (Clayton and George, 1999). Deletions or point mutations in the *parkin* gene, which transcribes a protein of unknown function, involved in the expression of autosomal recessive juvenile-onset Parkinsonism have also recently been identified in patients of

North African, European and Japanese descent (Hattori et al., 1998; Kitada et al., 1998; Leroy et al., 1998; Abbas et al., 1999; Shimura et al., 1999). However, alterations of α-synuclein or parkin expression have not been found to be universal to all PD cases (Parsian et al., 1998; Wang et al., 1998; Lin et al., 1999; Scott et al., 1999; Shimura et al., 1999).

In 1983, Langston and colleagues reported on the sudden onset of PD-like symptoms in a group of 4 catatonic patients in California (Langston et al., 1983). Upon further investigation it was discovered that each of these patients had developed Parkinsonism following self-administration of a designer drug (Langston et al., 1983). In the production of that designer drug, a neurotoxic side-product had formed, 1-methyl-4-phenyl-tetrahydropyridine (MPTP). MPTP was discovered to be a selective neurotoxin, destroying the DAergic neurons within the nervous system and has since been used in the production of animal models of PD. When this neurotoxin was identified, researchers hypothesized that PD may develop after years of exposure to environmental toxins. Many studies have investigated whether there may be regional differences in PD distribution or more specifically, whether there was an increased incidence of PD in relation to a particular geographical region, occupation, drinking water, as well as other possible factors (Semcuk et al., 1995; Seidler et al., 1996; Marder et al., 1998; Gorell et al., 1999). Thus far, the data obtained in those studies remains inconclusive as to whether there may be a strong correlation between any of those factors and the incidence of PD. Until the cause of PD is known, many researchers are studying ways in which to lessen the degree of disability in this patient population. The most predominant treatment modality currently in use is pharmacological therapy using L-Dopa.

Pharmacological treatment of PD

Since the 1960's, the mainstay treatment for PD has involved the elevation of DA levels through the administration of Sinemet®, a combination of L-Dopa and carbidopa. L-Dopa, unlike DA readily crosses the blood brain barrier where it is enzymatically converted to DA by DOPA decarboxylase (DDC) within the remaining DAergic neurons and carbidopa inhibits DDC activity within extracerebral regions (Kopin, 1994). L-Dopa administration is initially effective in relieving bradykinesia, rigidity and tremor, the main symptoms in PD, however the clinical benefits of L-Dopa decrease with long-term use and side effects often develop, which include dyskinesias and motor fluctuations, the "on-off phenomenon" (Marsden and Parkes, 1977). With time, the length of time in the 'off' phase, a period of akinesia, increases and the 'on' phase, a period of activity, decreases and is associated with severe dyskinetic or abnormal movements (Marsden and Parkes, 1977). That reduction in clinical efficacy results from the continued progression of the disease and further degeneration of the DAergic SNc neurons. Furthermore, evidence has been compiled to suggest that L-Dopa may increase disease progression by increasing DA toxicity, where free radicals are generated through the autoxidation of DA (Mena et al., 1992; Chiueh et al., 1994; Smith et al., 1994; Pardo et al., 1995).

Several other pharmacological treatments are currently being investigated clinically, to slow the progression of the disease process or treat Parkinsonism. Selegiline® or deprenyl, a monoamine oxidase type B (MAO_B) inhibitor, is effective in slowing the progression of the disease, increasing the time between positive diagnosis and requirement of L-Dopa treatment, and in providing some amelioration of Parkinsonian deficits (Parkinson Study Group, 1989; 1993; Tetrud and Langston, 1989;

Alain et al., 1991; LeWitt and the Parkinson Study Group, 1991; Lieberman and Fazzini, 1991; Myllylä et al., 1991; 1992; Brannan and Yahr, 1995;). Aside from deprenyl's ability to enhance DA levels in the synaptic cleft, evidence has been compiled suggesting that deprenyl may also be neuroprotective, independent of its ability to inhibit MAO_B and the degradation of DA (Ansari et al., 1993; Tatton, 1993; Ju et al., 1994; Mytilineou et al., 1997), possibly by suppressing the generation of free radicals and oxidative stress or by inducing superoxide dismutase activity (Cohen and Spina, 1989; Salo et al., 1992; Chiueh et al., 1994; Gerlach et al., 1994; Kitani et al., 1996; Tatton and Chalmers-Redman, 1996; Wu et al., 1996). Deprenyl has also been observed to enhance ciliary neurotrophic factor expression by astrocytes (Seniuk et al., 1994). Catabolism of deprenyl in the liver liberates methamphetamine (Karoum et al., 1982; Engberg et al., 1991; Sziráki et al., 1994), which enhances DA neurotransmission and may also provide some clinical benefit. Administration of deprenyl does not prevent the progression of the disease or the eventual need for L-Dopa therapy. The ability of other drugs to alleviate Parkinsonian symptoms is presently being clinically investigated.

Other pharmacological therapies currently being evaluated in PD can be divided into 3 main categories: glutamate and acetylcholine antagonists, and DA agonists. Antagonists of the N-methyl-D-aspartate (NMDA) glutamate receptor have been observed to be effective in reducing L-Dopa-induced dyskinesias and motor fluctuations (Verhagen-Metman et al., 1998a; 1998b). Although no serious side effects in those studies have been reported, further long-term studies are needed to evaluate the safety and efficacy of NMDA antagonists. Anti-cholinergics have been successful in reducing tremor but not akinesia or rigidity (Wasielewski et al., 1998; Schrag et al., 1999).

Furthermore, anticholinergic treatment has been linked to the appearance of dementia in PD patients (Nishiyama et al., 1993; Pondal et al., 1996). The ergot derivatives (bromocriptine, lisuride, pramipexole, ropinirole and pergolide) are agonists of the D_2 DA receptor and are effective in alleviating Parkinsonian symptoms, but unfortunately for only short periods of time (1 year or less) in the majority of cases (Pezzoli et al., 1995; Bayulkem et al., 1996; Guttman, 1997; Lieberman et al., 1997; 1998; Alarcon et al., 1998; Brooks et al., 1998; Korczyn et al., 1998; Barone et al., 1999). No severe side effects were noted. Unfortunately, bromocriptine does not provide long-term benefit and in more advanced PD cases, the simultaneous treatment of PD with L-Dopa and bromocriptine affords no significant benefit over L-Dopa treatment alone (Alarcon et al., 1998). Furthermore, the administration of apomorphine (a D_1/D_2 DA receptor agonist) in low doses has been observed to significantly ameliorate PD symptoms for up to 66 months, however the quality of 'off' time and the intensity of dyskinesias remain unaffected (Pietz et al., 1998; Ondo et al., 1999).

Overall, the above treatments are effective to varying degrees in treating PD or slowing disease progression with some drugs being more effective than others in alleviating specific subsets of parkinsonian symptoms. In the case of bromocriptine, clinical benefits are short-lived. The administration of the other ergot derivatives, apomorphine or NMDA antagonists are still in the early stages of clinical trials and further long-term studies of their effectiveness and safety in PD patient's are required. Therapeutic strategies involving anticholinergics and L-Dopa quite often lead to the emergence of adverse side effects with long-term use. Currently, neurosurgical strategies are receiving increased attention for the possible treatment of PD. Several different

strategies are currently being investigated. To fully understand the rationale for the various neurosurgical techniques being tested, a brief review of the anatomy and physiology of the basal ganglia in normal and Parkinson states follows.

Basal ganglia anatomy and physiology in normal and Parkinson states

The striatum is comprised of the caudate nucleus and the putamen and receives topographically-organized motor-related inputs from the motor, supplementary motor, association motor and somatosensory cortices as well as the frontal eye fields (Kunzle 1975; 1977; Kunzle and Akert, 1977; DeLong et al., 1986). However, the motor cortices innervate several other basal ganglia nuclei as well. The motor, premotor and somatosensory cortices project predominantly to the putamen, and the association cortices to the caudate nucleus (DeLong et al., 1986). The descending glutamatergic corticostriatal projections are excitatory upon striatal neurons (Kaneko & Mizuno, 1988), which in turn provide γ-aminobutyric acidergic (GABAergic) efferent innervation of both internal (GPi) and external segments (GPe) of the globus pallidus (Loopujit and Van Der Rooy, 1985; Rajakumar, et al., 1994) and the substantia nigra (Loopujit and Van Der Rooy, 1985).

PD pathology is marked by degeneration of the nigrostriatal pathway comprised of the DAergic substantia nigra pars compacta (SNc) neurons projecting to the striatum. The SNc receives excitatory innervation from motor and frontal cortices (Carter, 1982; Schmidt, 1995) along with inputs from the amygdala, dorsal raphe nucleus (Vertes, 1991) laterodorsal and pedunculopontine tegmental nuclei (Clarke et al., 1987; Gould et al., 1989; Semba and Fibiger, 1992; Lavoie and Parent, 1994). DA released from the

nigrostriatal axons, binds to D_1 and D_2 receptors in the striatum. D_1 receptors are predominantly located upon striatonigral neurons, whereas D_2 receptors are localized to striatopallidal neurons (Robertson, 1992a; Robertson et al., 1992). Stimulation of striatonigral D_1 receptors induces the release of GABA within the substantia nigra (Robertson, 1992a; O'Conner, 1998). Stimulation of D_2 receptors on the tonically-active striatopallidal neurons inhibits the release of GABA within the GPi and GPe (Loopujit and Van Der Rooy, 1985; Rajakumar et al., 1994; O'Connor, 1998) (Figure 1.1). The selective death of the DAergic projections upon the striatum would effectively abolish the inhibitory drive of the striatum upon the substantia nigra and prevent the inhibition of striatopallidal neurons (Figure 1.2).

Within the indirect pathway (striatum → GPe → STN → GPi → thalamus), both projections from the GPe to the STN and GPi to the thalamus are inhibitory upon the excitatory glutamatergic neurons comprising those nuclei (Kaneko and Mizuno, 1988; Smith and Parent, 1988; Schmidt, 1995). An interesting and important feature of the basal ganglia-motor circuit arises when considering the inhibitory pallidothalamic and pallidosubthalamic projections. The tonically-active GABAergic neurons projecting from the striatum to the GPi and GPe inhibit the GABAergic neurons of those structures under resting conditions. This in turn leads to the increased excitatory drive of the subthalamic nucleus upon the GPi and increased inhibition of thalamocortical efferents (Schell and Strick, 1984; Nambu et al., 1988). Within the direct pathway (striatum → SNr → thalamus), striatonigral efferents remain underactive and the tonically-active nigrothalamic pathway continues to have an inhibitory influence upon the thalamus (Kilpatrick et al., 1980; MacLeod et al., 1980; Kemel et al., 1988). During periods of

voluntary movement, D_2 stimulation inhibits the striatopallidal pathway disinhibiting pallidothalamic and pallidosubthalamic pathways, allowing for the release of GABA within the subthalamic nucleus via GPe projection neurons. Stimulation of the striatonigral pathway would enhance GABA transmission within the SN, releasing the inhibitory drive of pars reticulata neurons upon the thalamus and other nuclei such as the superior colliculus, reticular formation and pedunculopontine tegmental nucleus (DiChiara et al., 1979; Kemel et al., 1988; Ficalora and Mize, 1989; Spann and Grofova, 1991; Bickford and Hall, 1992; Yasui et al., 1996). Overall, the net effect would be that the tonically-active thalamocortical neurons would be released from inhibition and free to fire upon cortical neurons allowing for the continuance of motion (Figure 1.1).

With the loss of the DAergic nigrostriatal efferents, the circuit closely resembles that of the resting condition even during periods of planned motor activity. With decreased striatal innervation by the SNc, inhibition of the SNr decreases and inhibition of the GPi and GPe remains (Figure 1.2). The constant inhibitory drive upon the GPe by the striatum results in the continual firing of STN neurons and thus, tonic excitation of GPi neurons, which in turn inhibit the thalamocortical projection neurons. Within the direct pathway there is decreased inhibition of tonically-active GABAergic nigrothalamic neurons, leading to increased inhibitory drive upon the thalamus. Thus, the overall activity of direct and indirect pathways has a net inhibitory drive upon the thalamus reducing excitation of motor cortical areas and preventing the continuance of motion (Albin et al., 1989) (Figure 1.2). This notion has been verified in Parkinsonian patients (Rascol et al., 1992). Aside from tonic inhibition of the STN by the GPe, recent evidence suggests that in PD patients there is also increased excitation of STN by the motor cortex

and parafascicularis nucleus of the thalamus (Levy et al., 1997), which would further enhance the excitatory influence of the STN upon the GPi.

An explanation for Parkinsonian tremor closely relates to the abnormal physiology of basal ganglia nuclei and their pathways. Subsets of thalamic (Lenz et al., 1995) and GPi neurons (Hutchinson et al., 1997) have a firing rate at tremor frequency in PD patients. The thalamic neurons lie anterior to the principal somatosensory nucleus, ventralis caudalis, in an area identified as the optimal site for placement of lesions to alleviate Parkinsonian tremor (Lenz et al., 1995).

The above model implicates the primary involvement of two structures in the hypokinetic/akinetic and rigidity characteristics of PD; the STN and GPi. Two surgical procedures have been incorporated to inactivate those overactive basal ganglia structures, ablation and high-frequency stimulation. Ablation and high-frequency stimulation of the thalamus has been observed to be mainly effective in treating tremor-dominant Parkinsonism. In contrast, elevation of striatal DA levels and reconstitution of basal ganglia anatomy and physiology through the transplantation of DA-rich tissue sources into the brains' of PD patients may offer the greatest opportunity of a cure for this debilitating disorder.

Figure 1.1 Simplified schematic representation of basal ganglia circuitry and activity during periods of active movement in humans. SNc = substantia nigra pars compacta; SNr = substantia nigra pars reticulata; GPi = internal segment of the globus pallidus; GPe = external segment of the globus pallidus; STN = subthalamic nucleus; D_1 and D_2 = D_1 and D_2 dopamine receptors; thick arrows = increased activity; thin arrows = reduced activity.

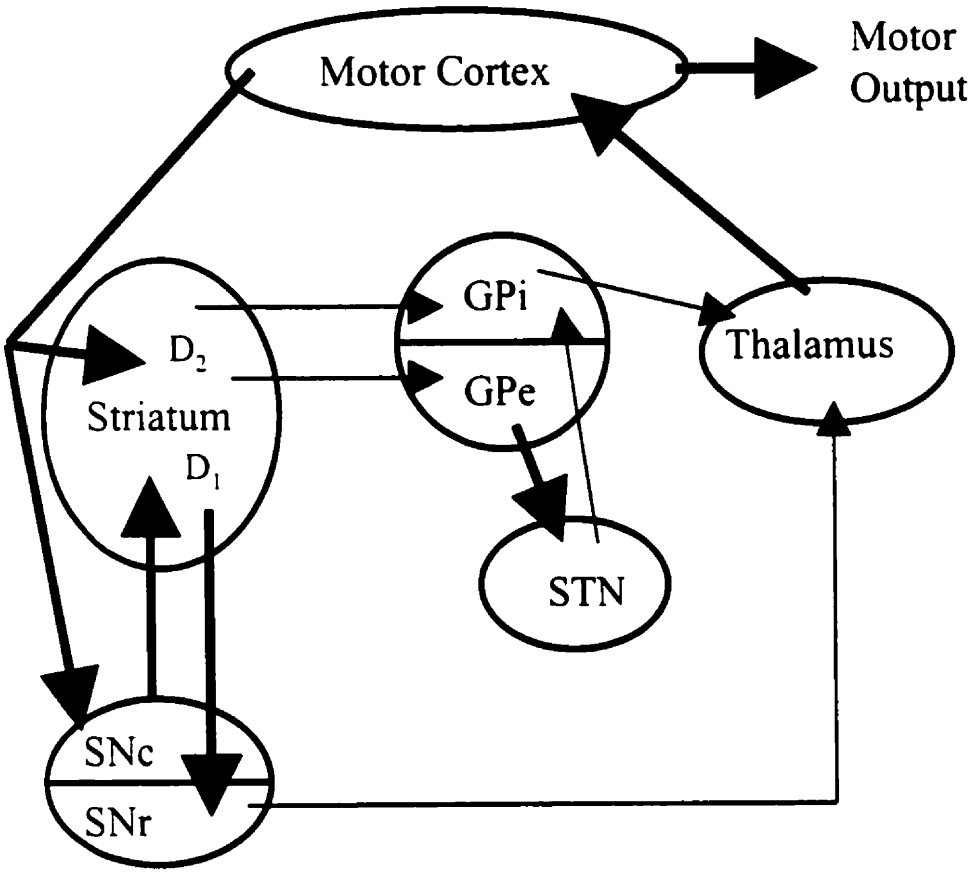
Motor Cortex
Motor
Output
GPi
Thalamus
D2
Striatum
GPe
D1
STN
SNc
SNr

Figure 1.1

Figure 1.2. Simplified schematic representation of basal ganglia circuitry and activity during periods of active movement in patients with Parkinson's disease. Broken arrows = degenerated nigrostriatal pathway; see the legend of Figure 1.1 for further explanation of abbreviations.

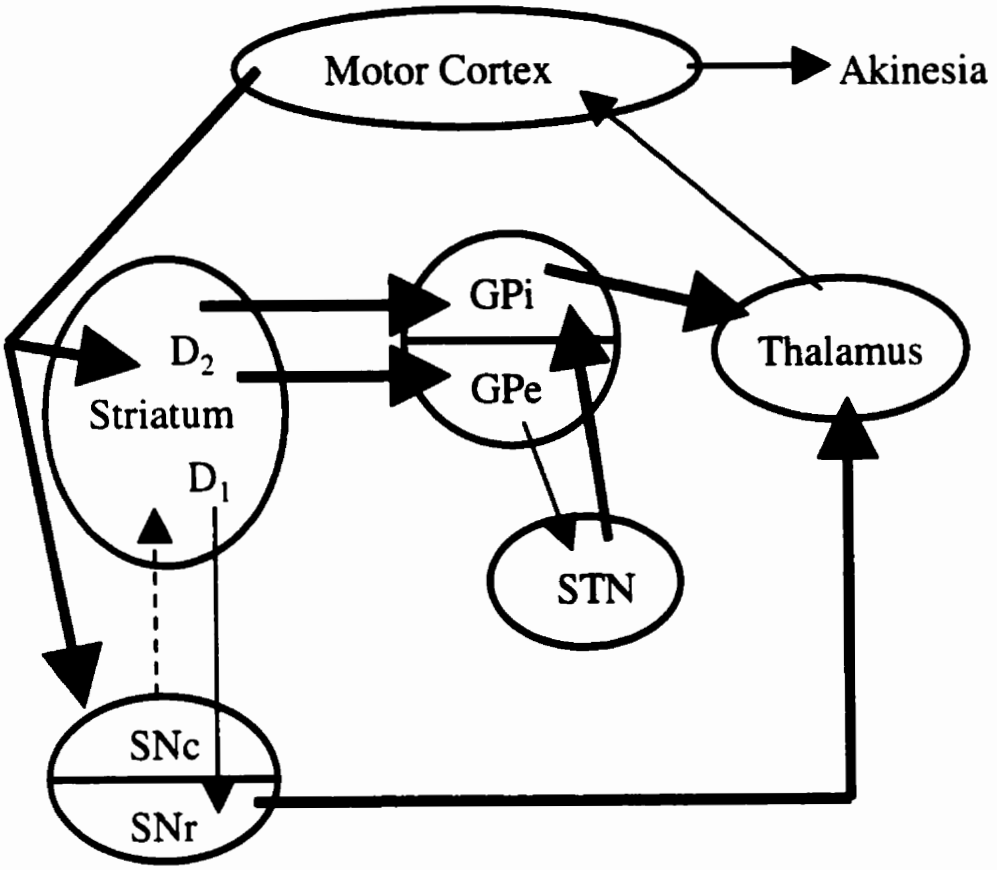
Motor Cortex
Akinesia
GPi
Thalamus
D2
Striatum
GPe
D1
STN
SNc
SNr

Figure 1.2

Neurosurgical strategies for the treatment of PD

Thalamic Lesions and Stimulation

Stereotactic thalamotomy was the main treatment for PD until the late 1960's and largely disappeared as a therapeutic option with the introduction of L-Dopa (Tasker et al., 1983). Thalamotomy is an ablative procedure that involves lesioning areas of the thalamus comprised of neurons displaying tremor-like bursting activity, primarily the ventral intermediate nucleus (Vim) or ventrolateral nucleus of the thalamus (Lenz et al., 1995) in PD patients. Ablation of the Vim appears to ameliorate Parkinsonian tremor. Tasker and colleagues reported in 1983 that Vim thalamotomy had abolished tremor in 82% of 75 subjects at 2 years postoperatively. There was no observed reduction in movement or speech deficits and only 7% of the patients presented persistent complications. One year later, Matsumoto and colleagues (1984) reported on the long-term follow-up (mean = 4.2 years) of 78 PD patients who underwent uni- or bilateral ventrolateral thalamotomy. In 44 of these patients, no progression of the disease was noted and in some patients there was a decrease in the L-Dopa dose administered. No results were reported by this group concerning the extent of postoperative tremor.

In more recent clinical trials, unilateral lesions of the Vim of the thalamus decreased contralateral tremor in 100% of patients at 3 months postoperatively (Boecker et al., 1997) and in 86% of patients upon a 13 year follow-up (Jankovic et al., 1995). In the latter study, the daily dose of L-Dopa administered had decreased in 35 of the 42 patients. In this study, postoperative complications were noted which included contralateral weakness, dysarthria, and confusion. Bilateral thalamotomies are associated with cognitive and speech disturbances and increased morbidity (Koller et al., 1997).

The high percentage of patients presenting immediate (58%) and persistent (23%) postoperative complications (Jankovic et al., 1995) demonstrates the need for an alternative treatment of Parkinsonism in patients with medically intractable tremor. An alternative to Vim thalamotomy is high-frequency thalamic stimulation, which reduces the frequency of postoperative complications by being reversible.

In 1987, Benabid and colleagues (1991) were the first to apply high-frequency electrical stimulation of the thalamus for the treatment of PD and observed a significant decrease in the severity of postoperative tremor. The mechanism by which high-frequency stimulation inactivates a brain structure remains unknown. Several recent studies have observed a significant decrease in tremor at 3 (Hubble et al., 1997; Koller et al., 1997), 6 (Defebvre et al., 1996) and 10 months (Pfann et al., 1996) and 1 (Koller et al., 1997) and 8 years (Benabid et al., 1996) following high-frequency stimulation of the Vim. Although the severity of postoperative tremor was reduced, thalamic stimulation has little beneficial effect on gait, bradykinesia or rigidity (Benabid et al., 1996; Defebvre et al., 1996). In one study, 31.6% of patients undergoing this procedure demonstrated minor reversible side effects (Benabid et al., 1996). In this same study, the researchers reported a 30% reduction in L-Dopa dosages as compared to preoperative levels. Thus, thalamic stimulation is a safer procedure than thalamotomy and is effective at reducing the frequency of tremor and is most beneficial for patients exhibiting a tremor-dominant form of PD.

Globus pallidus lesions and stimulation

In Sweden during the 1950's, Lars Leksell improved upon an experimental

surgical technique used in the 1930's for the treatment of PD, known as pallidotomy (Laitinen et al., 1992). This surgical procedure involves inactivation of the globus pallidus, which exhibits abnormal physiological activity in the Parkinsonian brain (Sterio et al, 1994). Early lesions of the posteroventral GPi by Leksell greatly reduced resting tremor, rigidity and bradykinesia in PD patients (Laitinen et al., 1992). Early pallidotomies by Leksell and others were met with a variety of side effects, including homonymous hemianopsia, transient dysphasia and transitory hemiparesis. Despite the dramatic alleviation of motor abnormalities, the side effects led to the abandonment of this technique as a therapeutic option for the treatment of Parkinsonism.

With the advent of more advanced technology (ie., imaging techniques) and improved surgical techniques (ie., computerized tomography-guided stereotactic surgery), the possible treatment of PD by pallidotomy has resurfaced (Laitinen et al., 1992). Since 1985, many PD patients have received this procedure for the treatment of their Parkinsonian symptoms. Many researchers have observed a dramatic improvement in speech and rigidity, and reduced time in the 'off' state as well as the severity and frequency of L-Dopa-induced dyskinesias up to 1 (Laitinen et al., 1992; Dogali et al., 1995; Iacono et al., 1995; Lozano et al., 1995; Sutton et al., 1995; Baron et al., 1996; Kishore et al., 1997; Kopyov et al., 1997a; Krauss et al., 1997; Lang et al., 1997; Soukoup et al., 1997) and 4 years (Fazzini et al., 1997) postoperatively. Pallidotomy has been demonstrated to only occasionally result in a reduction of anti-Parkinsonian medications and is less effective than thalamotomy for the treatment of tremor-dominant PD (Tasker et al., 1997), although Laitinen and colleagues (1992) reported an almost complete abolishment of tremor in 81% of patients.

Stimulation of the GPi for Parkinsonian symptoms has recently been incorporated as an alternative to GPi pallidotomy for the treatment of this disease. Pallidal stimulation involves high-frequency stimulation of the venteroposterolateral GPi which effectively inactivates this structure, in comparison to low-frequency stimulation which results in enhancement of motor symptoms (Gross et al., 1997). As mentioned above, GPi pallidotomy is occasionally met with varying transient and long-term side effects due primarily to the close proximity of the optic tract to the GPi (Laitinen et al., 1992; Hariz and DeSalles, 1997). GPi stimulation is beneficial by being reversible in that the microelectrode can easily be repositioned if incorrectly placed. This was demonstrated by Gross and colleagues (1997), where a patient reported a transient flash of light when the stimulator was turned on, suggesting incorrect positioning of the electrode near the optic tract.

In the clinical studies reported thus far there has been a demonstrated reduction in akinesia, rigidity, as well as decreased gait and speech disturbances up to 3 years postimplantation (Siegfried and Lippitz, 1994; Davis et al., 1997; Gross et al., 1997; Limousin et al., 1997; Pahwa et al., 1997; Tronnier et al., 1997). Gross and colleagues (1997) reported a decrease in tremor in 4 of 5 patients up to 3 years postimplantation, whereas other groups failed to report any changes or reduction in the frequency of tremor (Davis et al., 1997; Limousin et al., 1997; Pahwa et al., 1997). A similar reduction in rigidity and akinesia has been reported in MPTP-treated monkeys following GPi stimulation (Boraud et al., 1996). Thus, it appears that GPi stimulation is similar to posteroventral pallidotomies in that both procedures inactivate the GPi and similarly reduce akinesia, rigidity and improve gait and speech disturbances. However, only GPi

stimulation is reversible.

Subthalamic nucleus

The results described above demonstrate that inactivation of the Vim is generally only effective in reducing the frequency and severity of tremor in PD patients, whereas inactivation of the ventroposterolateral GPi is generally ineffective in reducing tremors but is effective in improving overall motor function. Those observations led to the identification of the STN as another possible important target site for inactivation. That hypothesis is supported by the observation that STN neurons display a tremor-like bursting frequency in PD patients (Rodriguez et al., 1998) and inactivation of that nucleus in MPTP-treated monkeys, offers significant alleviation of tremor and an overall enhancement of motor function (Wichmann et al., 1994; Guridi et al., 1996). Those findings have led to clinical trials into the ability of subthalamic inactivation in treating parkinsonism. The results reported thus far, indicate that either subthalamotomy or high-frequency subthalamic stimulation is effective in improving overall motor function and reducing tremor and required L-Dopa dosages up to 18 months postimplantation (Krack et al., 1997a; 1997b; 1998a; 1998b; Kumar et al., 1998a; 1998b; 1999; Limousin et al., 1998; Brown et al., 1999; Moro et al., 1999; Yokoyama et al., 1999). Thus, inactivation of the STN appears to be a technique that can be applied to a wide range of PD patients, however this technique is only in its infancy and further studies are required to evaluate the efficacy of STN inactivation in treating parkinsonism over longer periods of time.

Neural transplantation

Pallidotomy / pallidal stimulation and thalamotomy / thalamic stimulation are surgical techniques that can only be applied to certain subsets of the PD patient population. Unfortunately, lesions within the STN are irreversible and the long-term effects of such lesions on patients have yet to be determined. On the other hand, subthalamic stimulation may be the favoured technique in the future as it is reversible, however over time the effectiveness of high-frequency stimulation in controlling parkinsonian symptoms may deteriorate as has occasionally been observed with GPi stimulation, resulting in frequent programming of the stimulator (Gross et al., 1997; Pahwa et al., 1997). The above techniques have proved somewhat effective in treating PD, however the greatest opportunity of a cure for PD may involve neurosurgical restoration of the nigrostriatal pathway.

Animal experiments and clinical studies

The first attempt to transplant neural tissue from one organism to another was reported in 1890 (Thompson, 1890). In 1890, Thompson reported on what he referred to as the "successful" transplantation of feline cerebral cortical tissue into the cortex of dogs. Unfortunately that study was done before the advent of immunosuppressive therapies and the cortical grafts failed to survive. The first description of surviving neuronal grafts following transplantation was reported in 1905 (Saltykow, 1905). It was reported that replantation of adult rabbit cortical autografts survived for up to 8 days (Saltykow, 1905). Twelve years later the first clear evidence of interanimal neuronal tissue survival was reported by Dunn (Dunn, 1917). In that study, rat neonatal cortical

tissue was transplanted into cavities created within the cortices of littermates. Dunn's study (1917) was important in providing some evidence that the age of the donor tissue and blood vascularization of the grafts may correlate with increasing cell survival. In 1940, the first attempt of fetal tissue transplantation was made (Le Gros Clark, 1940). In that study, Le Gros Clark suggested that, fetal grafts may have the best potential to reestablish host architecture. The notion that transplanted neural tissue is capable of innervating adjacent tissue was demonstrated by May (1949). In that study May (1949) cotransplanted muscle and cerebellar tissue in the anterior chamber of the mouse eye. Grafted cerebellar fibers were clearly seen to innervate adjacent mucle tissue (May, 1949). Halasz and colleagues (1963; 1965) later reported that transplantation of pituitary gland within the medial basal hypothalamus of hyophysectomized rats reversed the endocrine deficits in those animals following hypophysectomy (Halasz et al., 1963; 1965). Those two studies were the first to demonstrate that grafted tissue has the ability to induce functional effects in the host animal. It was well-established by the late 1970's that fetal grafts integrated well within the host brain, reconstituted damaged pathways and reinnervated denervated regions of the brain (Das, 1974; Björklund et al., 1976; 1979; Björklund & Stenevi, 1977; 1979; Lund and Hauscha, 1979). The positive results obtained in those experiments, provided the framework for applying fetal tissue transplantation to animal models of neurodegenerative disorders.

By the early 1980's, many studies reported the recovery of sensorimotor deficits following transplantation of fetal DAergic tissue in the DA-depleted striatum of the 6-hydroxydopamine (6-OHDA) rat model of PD (Perlow et al., 1979; Björklund et al., 1980; Freed et al., 1980; Dunnett et al., 1981a; 1981b; 1981c; 1983a; 1983b). The study

by Perlow and colleagues (1979) demonstrated that DAergic tissue was required for functional benefit, as intrastriatally grafted sciatic nerve afforded no significant benefit. Furthermore, they also reported for the first time, the long-term survival and functional benefits of intrastriatal fetal nigral grafts (10 months) in the lesioned rat. During this period, Freed and colleagues (1981) reported that intrastriatal grafts of adrenal medullary (AM) tissue as a source of catecholaminergic cells also produced functional benefit in the rat model of PD although graft survival was poor. The main rationale for using AM tissue in neural transplantation was that the tissue could be harvested from the patient's own body, thereby circumventing the ethical issues and the need for immunosuppressive therapies that accompany fetal tissue use.

The first attempts at neural transplantation in PD sufferers involved the use of AM tissue. The first transplantation of autologous AM cells into the caudate nucleus of a parkinsonian patient was carried out in Sweden in 1982. Although no serious side effects were noted, only moderate motor benefits were observed and only for a short period of time (Backlund et al., 1985). In a subsequent study, only minor improvements in motor function were again exhibited by two PD patients with intraputaminal AM autografts (Lindvall et al., 1987). The most dramatic alleviation of parkinsonian symptoms following unilateral transplantation of an AM autograft into the striatum was reported by Madrazo and colleagues (1987). At 5 months posttransplantation, all anti-parkinsonian medications were discontinued in one patient, rigidity and akinesia were absent bilaterally and tremor was significantly attenuated. Although those findings attracted great interest, 11 patients had received AM autografts but only the results of 2 patients were actually reported. Of the remaining 9 patients, 2 had died with no evidence of graft

survival (Mehta et al., 1997). Following those positive results published in 1987, many more trials were conducted into the efficacy of AM autografts in treating parkinsonism, but none of those studies were able to replicate the findings of Madrazo and colleagues (Allen et al., 1989; Bakay, 1989; Goetz et al., 1989; 1991; Kelly et al., 1989; Apuzzo et al., 1990; Flores, 1990). In 1989, a study comparing AM grafts to fetal nigral grafts was conducted in the rat model and demonstrated that grafted nigral cells exhibited better survival and provided longer-lasting functional benefit than AM grafts (Brown and Dunnett, 1989). In a study published a year later, AM cells were reported to switch from their catecholaminergic phenotype *in vivo* (Waters et al., 1990). Those results largely led to the abandonment of clinical studies evaluating the efficacy of AM autografts in PD. Neural transplantation research then shifted its focus to evaluating the feasibility of using fetal nigral grafts to alleviate parkinsonism.

Since the early studies on fetal nigral transplantation in the rat model of PD, many studies have demonstrated the survival of intrastriatal DAergic grafts within the host brain, restoration of DA agonist-induced rotational asymmmetry (Björklund et al., 1980; Dunnett et al., 1981b; 1986; Brundin et al., 1988; Robertson et al., 1991; Nikkhah et al., 1993; Olsson et al., 1995; Mendez et al., 1996; Apostolides et al., 1998), reinnervation of the DA-depleted striatum by graft-derived THir fibers (Björklund et al., 1980; Dunnett et al., 1981a; 1981b; 1981c; Björklund et al., 1983; Brundin et al., 1988; Rioux et al., 1991; Mendez et al., 1996; Apostolides et al., 1998; Mehta et al., 1998) and the formation of synaptic contacts between those fibers and host striatal neurons (Freund et al., 1985; Clarke et al., 1988; Nishino et al., 1990; Mendez et al., 1991; 1992). Furthermore, normalization of DA levels in the striatum (Schmidt et al., 1983; Nishino et al., 1990; Moukhles et al., 1994; Reum and

Morgenstern, 1994; Earl et al., 1996; Hashitani et al., 1998), and partial to complete reversal of biochemical deficits is often observed in striatal neurons following intrastriatal nigral transplantation (Cadet et al., 1991; Segovia et al., 1991; Sirinathsinghji and Dunnett, 1991; Mendez et al., 1992; 1993; Bal et al., 1993; Cenci et al., 1993; Abrous et al., 1994; Zeng et al., 1996).

Although those studies are very promising, the rat model of PD does not closely resemble the human parkinsonian condition. A non-human primate model of PD was developed with symptoms more closely resembling the human condition. MPTP-treated parkinsonian monkeys develop the cardinal symptoms of PD: bradykinesia, tremor and rigidity. Thus an animal model was available more closely resembling the human condition in which to possibly evaluate the safety and efficacy of fetal nigral tissue transplantation in non-human primates. In the mid-1980's, a few studies were published suggesting that transplantation of fetal DAergic tissue to the striatum of MPTP-treated monkeys provided significant improvement in parkinsonian symptoms (Redmond Jr et al., 1986; Sladek Jr et al., 1986; 1987; 1988) and provided a strong rationale for in which to commence clinical trials in human PD patients.

In 1987, the first report of fetal tissue transplantation in a PD patient was published (Jiang et al., 1987). Since that first report over 200 patients have since received fetal nigral transplants in several clinics around the world (Hitchcock et al., 1988; Lindvall et al., 1988; 1989; 1990; 1992; 1994; Madrazo et al., 1988; 1990a; 1990b; Freed et al., 1990; 1992; Henderson et al., 1991; Spencer et al., 1992; Widner et al., 1992; Peschanski et al., 1994; Wu et al., 1994; Freeman et al., 1995; 1997; Kordower et al., 1995; 1996; 1997; López-Lozano et al., 1995; 1997; Defer et al., 1996; Kopyov et al., 1997b; Levivier et al., 1997;

Wenning et al., 1997; Lindvall, 1998; Bluml et al., 1999; Hagell et al., 1999; Hauser et al., 1999). In 1988, Lindvall and colleagues reported on their experience with the transplantation of 8-10 week old fetal ventral mesencephalon (FVM) in the caudate nucleus and putamen of 2 immunosuppressed patients with advanced Parkinson's disease (Lindvall et al., 1988). In that trial, the patients exhibited improved speed of movement and motor readiness potentials. Postiron emission tomography (PET) scans demonstrated a slight increase in flourodopa uptake in the grafted striatum 1-year posttransplantation, suggesting survival of the DAergic graft (Lindvall et al., 1989). Lindvall and colleagues made minor adjustments to the transplantation technique and in a subsequent study reported a significant improvement in rigidity, reduced time spent in the "off" period and increased fluorodopa uptake as evidenced on PET scans, 8 months following transplantation (Lindvall et al., 1990).

In 1992, three important studies were reported demonstrating functional benefit and survival of DAergic grafts. In the first study, Spencer and colleagues (1992) transplanted cryopreserved 7-11 week gestational age solid fragments of fetal tissue unilaterally into the caudate nucleus of 4 immunosuppressed PD patients. In that study, it was reported that there was significant bilateral improvement in motor tasks and activities of daily living (ADL) by the patients. In the second study, Freed and colleagues (1992) transplanted 5 patients bilaterally with solid fetal nigral grafts and 2 others received unilateral grafts, with every other patient receiving immunosuppressive therapy. All 7 patients reported improvement in ADL functions and 5 showed improvement on the neurological exam and at least 1 patient exhibited a significant increase in fluorodopa uptake at 46 months following grafting. In the third study, Widner and colleagues (1992) reported on the results of 2 bilaterally

transplanted patients (6-8 weeks gestational age VM tissue) with MPTP-induced parkinsonism. In those patients there was a significant improvement in motor function and increased fluorodopa uptake.

Another important study was published by Freeman and colleagues in 1995. Four PD patients received bilateral transplants of fetal nigral tissue in the putamen. A significant enhancement of performance on the Unified Parkinson's Disease Rating Scale and fluorodopa uptake on PET scans were reported. The most compelling evidence for graft survival and striatal reinnervation was reported following the unfortunate death of one of the patients from causes unrelated to the transplantation surgery, 18 months following transplantation (Kordower et al., 1995; 1996; 1997). Immunocytochemical analyses of the brain, revealed that there were more than 200.000 surviving THir cells within the graft and extensive reinnervation of the host putamen in a patch-matrix fashion by THir fibers. Electron microscopy revealed numerous synaptic contacts between grafted and host neurons. Sustained clinical recovery and continued reductions in L-Dopa dosages administered have been reported for more than 5 years posttransplantation (López-Lozano et al., 1997; Wenning et al., 1997; Hagell et al., 1999).

Issues to be resolved in neural transplantation

Although the clinical findings thus far are promising, neural transplantation remains an experimental procedure. Several issues have been identified that need to be resolved before neural transplantation can be considered a routine therapeutic procedure for the treatment of PD (Olanow et al., 1996; Mehta et al., 1997). Generally, these issues include the relative short supply of fetal tissue and the optimal tissue age for transplantation.

Furthermore, as with any type of human-human transplant, there is always the risk of disease transmission and graft immunorejection. Based on animal studies, the appropriate age of the donor tissue is generally known (Simonds and Freed, 1990; Brundin et al., 1986; Kondoh et al, 1996; Annett et al., 1997) and the probability of graft rejection can be lessened with chronic administration of immunosuppressants (Brundin et al., 1988). However, there is serious concern and questions surrounding the duration of time the immunosuppressive therapy should be maintained following transplantation, as the aging patient is vulnerable to infection and the immunosuppressant itself (Cyclosporine-A) has harmful side effects (Bennett, 1998). The risk of disease transmission is minimal as the tissue is carefully screened for viral pathogens prior to transplantation (Mehta et al., 1997). Although the above issues are generally well controlled the relative short supply of suitable tissue for transplantation limits the likelihood of neural transplantation to be incorporated as a routine treatment strategy for neurological disorders. There is also poor survival of DAergic cells in the host brain following transplantation (Björklund et al., 1980; Dunnett et al., 1981a; 1981b; Apostolides et al., 1998; Mehta et al., 1998). Although increasing DAergic neuron survival is crucial for clinical efficacy in PD patients, the optimal target site(s) for DAergic neurons to produce maximal clinical benefit also needs to be determined. Furthermore, several important issues concerning the transplantation procedure itself also need to be addressed.

Relative short supply of fetal tissue

One way to address the issue of a short supply of fetal tissue for transplantation would be to enhance the survival of grafted DAergic neurons. Evidence from our laboratory

and other investigators have explored the ability of glial cell line-derived neurotrophic factor (GDNF) and the GDNF-related molecule, neurturin (NTN) to promote the survival of grafted dopaminergic cells (Rosenblad et al., 1996; 1999; Sinclair et al., 1996; Granholm et al., 1997; Apostolides et al., 1998; Mehta et al., 1998; Sautter et al., 1998b; Sullivan et al., 1998; Yurek, 1998; Wilby et al., 1999). In all of those studies, exposure of grafted DA neurons to GDNF or NTN significantly enhanced cell survival. Similarly, brain-derived neurotrophic factor (BDNF), insulin-like growth factor-1, basic fibroblast growth factor (bFGF) or combinations of the above have been shown to enhance the survival of nigral DAergic neurons in culture and/or following transplantation (Steinbusch et al., 1990; Mayer et al., 1993; Takayama et al., 1995; Zeng et al., 1996; Thajeb et al., 1997; Sautter et al., 1998a; Zawada et al., 1998). It has also been considered that the grafted neurons may die from increased intracellular concentrations of reactive oxygen species or by an apoptotic mechanism. Prior exposure of nigral neurons to anti-oxidant and/or anti-apoptotic molecules also increases the survival of nigral neurons *in vitro* and/or *in vivo* (Nakao et al., 1994; Grasbon-Frodl et al., 1996; Othberg et al., 1997; Schierle et al., 1999). Although all of the above factors promote the survival of nigral neurons *in vitro* and/or *in vivo*, a reliance on fetal tissue is likely to be a major obstacle for the expansion of neural transplantation as a therapeutic strategy for PD.

Finding an alternative tissue source to fetal-derived tissue for transplantation in PD is of major importance. Many studies have been conducted to investigate the ability of genetically-engineered cells of both neuronal and non-neuronal origin to overexpress TH in promoting functional recovery in the rat Parkinson model (Wolff et al., 1989; Horellou et al., 1990a; 1990b; Fisher et al., 1991; Ishida et al., 1996a; Lundberg et al., 1996;

Tornatore et al., 1996; Raymon et al., 1997; Leff et al., 1998; Fitoussi et al., 1998; Segovia et al., 1998). Although, grafts of genetically-engineered cells inititially promote functional recovery, this effect is short lasting as host cells transfected with various genes often down-regulate expression of the foreign transgene (Palmer et al., 1991; Schinstine et al., 1992; Leff et al., 1998; Lundberg et al., 1996). One way that experimenters may be able to promote longer term expression of the foreign gene is for the transgene to be linked to the promoter of a constitutively expressed protein (Schinstine et al., 1992; Fisher et al., 1993; Tai and Sun., 1993; Trejo et al., 1999). Although this approach may provide some functional benefit a better strategy may be the transplantation of neuronal cells capable of reinnervating the denervated striatum.

Xenografts of porcine-derived FVM tissue have been observed to survive in the neostriatum of PD patients for up to seven months (Deacon et al., 1997). Intrastriatal grafts of fetal porcine tissue in 6-hydroxydopamine (6-OHDA)-lesioned rats have also been shown to survive, provide functional benefit and reinnervate the host striatum (Isacson et al., 1995; Galpern et al., 1996; Isacson and Deacon, 1996; Dinsmore et al., *in press*). Although these results are promising, there is great concern over the possibility of interspecies disease transmission (Isacson and Breakefield, 1997; Butler, 1998).

An exciting discovery for the field of neural transplantation has been the isolation of stem cells in the adult brain. Stem cells are self-renewing and can be induced to proliferate *in vitro* by exposure to mitogens such as, epidermal growth factor and differentiate into neuronal and glial cell phenotypes following mitogen withdrawal and exposure to the appropriate substrate and/or neurotrophic factors (Reynolds and Weiss, 1992; Weiss et al., 1996). The ability of stem cells to proliferate in culture is promising,

as stem cells could provide a readily abundant supply of tissue for transplantation. Stem cells have been observed to survive transplantation into the host brain (Svendsen et al., 1996; 1997; Lundberg and Björklund; 1996; Olsson et al., 1997; Deacon et al., 1998; Studer et al., 1998; Zigova et al., 1998). However, the behavioural recovery in animal models of PD following intrastriatal transplants are variable (Svendsen et al., 1997; Studer et al., 1998), which may relate to their low levels of TH expression *in vivo* (Svendsen et al., 1996; 1997; Deacon et al., 1998). Thus, the future of these cells as an alternative for transplantation relies on our ability to produce stem cell lines capable of stably expressing a DAergic phenotype (Studer et al., 1998; Wagner et al., 1999).

Other alternative cell lines that have been investigated include neuronal cells derived from brain tumours expressing a DAergic phenotype. Those studies demonstrated a significant reduction in DA agonist-induced rotational asymmetry (Hefti et al., 1985; Bing et al., 1988; Manaster et al., 1992; Tresco et al., 1992; Adams et al., 1996; Emerich et al., 1996). However, poor graft survival is commonly seen and there is always a concern that those cells may revert to a neoplastic state (Hefti et al., 1985; Bing et al., 1988). A recent promising development has been the discovery of a cell line derived from a human teratocarcinoma or germ cell tumor, with neuron-like properties (hNT) (Andrews, 1984; 1987; Lee and Andrews, 1986; Abraham et al., 1991; Pleasure et al., 1992; Pleasure & Lee, 1993). The ability of those neurons to survive, express a DAergic phenotype and promote functional recovery in the rat model of PD has been the focus of the first part of my studies (Chapter 2).

Optimal graft placement

Evidence for the growth and survival of intrastriatal DAergic grafts and their reversal of biochemical and locomotor deficits in animal models of Parkinson's disease (PD) is well documented. However, intrastriatal DAergic grafts fail to provide a complete alleviation of symptoms in PD patients. Furthermore, intrastriatal grafts do not fully alleviate complex sensorimotor deficits in the rat model (Nikkhah et al., 1993; Olsson et al., 1995; Mehta et al., 1998; Winkler et al., 1999) and restoration of the nigrostriatal pathway has not been achieved by the current grafting strategy. To date, the main transplant strategy has been to place nigral grafts not in their ontogenic site (substantia nigra) but in their target area (striatum). However, restoration of nigrostriatal circuitry with DAergic neurons and their dendrites in the SN and terminals in the striatum may be essential for more complete alleviation of the variety of symptoms in PD (Robertson, 1992b). Previously it has been demonstrated that DA is released from dendrites of SNc neurons in the SNr (Cheramy et al., 1979; 1981). This dendritic release of DA is thought to be important in enhancing GABA release through D_1 DA receptors localized to the descending striatonigral fibers in the SNr (Robertson, 1992a), reducing GABA transmission in the ventromedial thalamus (Gauchy et al, 1987) and increasing locomotor activity (Jackson and Kelly, 1983a; 1983b).

Those observations suggest that the SN itself may be an important target site for transplantation and intranigral DAergic grafts. Our laboratory has previously observed a significant reduction in amphetamine-induced rotational behaviour in the rat model of PD with simultaneous intrastriatal and intranigral DAergic grafts (double grafts) (Mendez et al., 1996). This reduction was superior to that of animals with intrastriatal grafts alone. Although, the greater functional recovery may be attributed to better modulation of basal

ganglia outflow, reinnervation of the SN may be crucial to improve graft-derived functional recovery in double grafted animals. The role of the intranigral graft in promoting functional recovery is the focus of the second part of my thesis (Chapter 3).

Figure 1.3 – A schematic diagram illustrating the dendritic release of DA and the projections of the SNr. GABA = γ-aminobutyric acid; PPT = pedunculopontine tegmental nucleus; RF = reticular formation; SC = superior colliculus; SNc = substantia nigra pars compacta; SNr = substantia nigra pars reticulata.

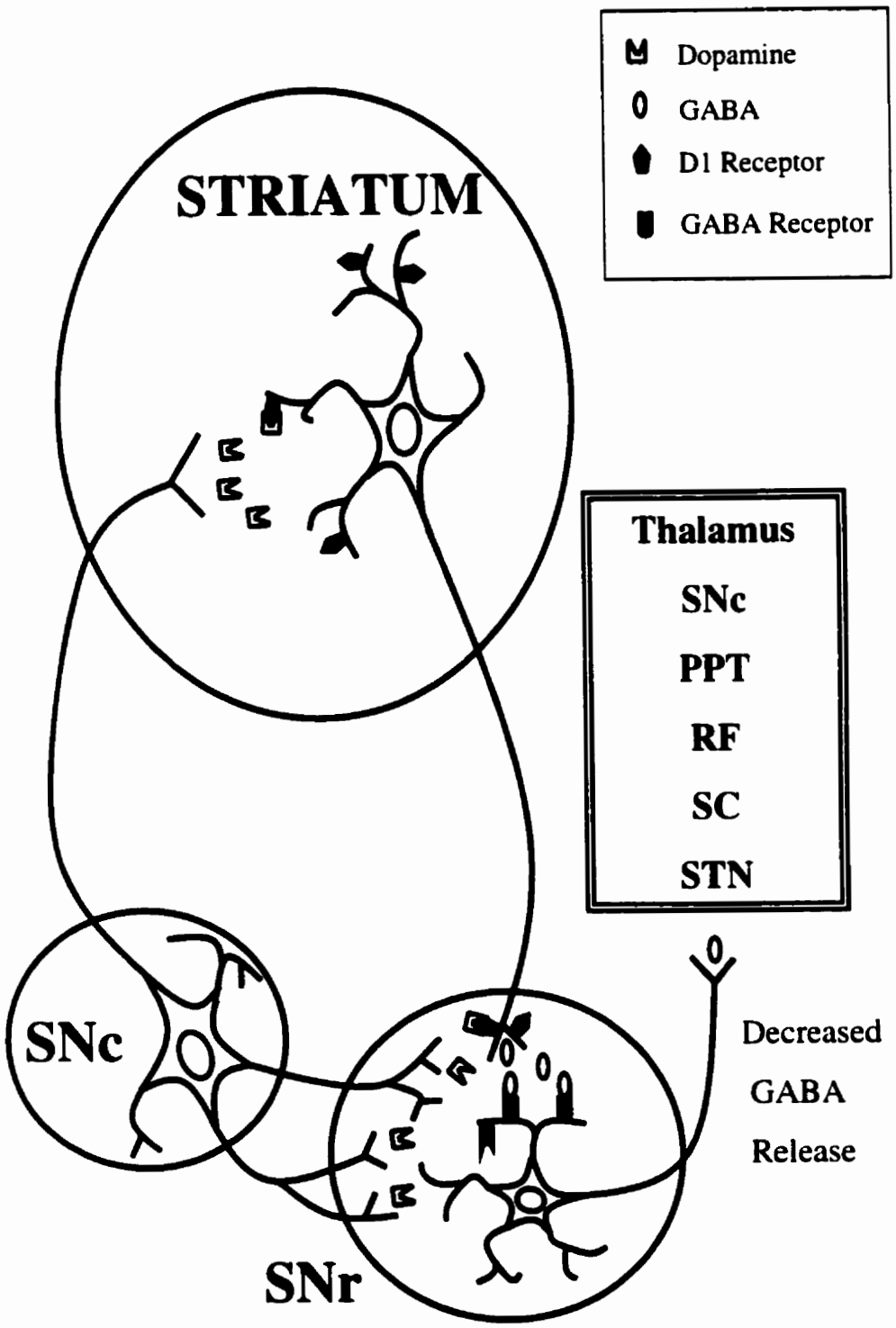
Dopamine
GABA
D1 Receptor
GABA Receptor
STRIATUM
Thalamus
SNc
PPT
RF
SC
STN
SNc
Decreased
GABA
Release
SNr
Increased GABA Release

Figure 1.3

CHAPTER 2:

INTRASTRIATAL AND INTRANIGRAL GRAFTING OF hNT NEURONS IN THE 6-OHDA RAT MODEL OF PARKINSON'S DISEASE

The results presented in the following chapter are currently in press in Experimental Neurology.

Introduction

The development of alternatives to fetal-derived cells for use in neural transplantation is of critical importance in the future of transplantation strategies for the treatment of neurodegenerative diseases such as PD. The ideal source of cells for the treatment of PD would be a limitless supply of DA-producing cells capable of reinnervating the host brain without the risk of immunorejection, disease transmission or tumour formation. Currently, a number of possible cell sources of both neuronal and nonneuronal origin are being studied. Xenografts of porcine-derived FVM tissue have been observed to survive in the neostriatum of PD patients for up to 7 months (Deacon et al., 1997). Intrastriatal grafts of fetal porcine tissue in 6-OHDA-lesioned rats have also been shown to survive, provide functional benefit, and reinnervate the host striatum (Isacson et al., 1995; Galpern et al., 1996; Isacson et al., 1996). Other researchers have focused on the development of genetically-engineered cell lines that overexpress TH (Wolff et al., 1989; Horellou et al., 1990a; 1990b; Fisher et al., 1991; Ishida et al., 1996b; Lundberg et al., 1996; Tornatore et al., 1996; Raymon et al., 1997; Fitoussi et al., 1998; Segovia et al., 1998) or neurotrophic factors that promote survival of DAergic cells (Levivier et al., 1995; Bilang-Bleuel et al., 1997). Despite these efforts, transplantation of genetically-engineered cells in animal models of PD has not provided conclusive long-term beneficial effects or reinnervation of the DA-depleted striatum. Another area that is currently being explored by a number of investigators including our own laboratory is the use of neural stem cells, which have the capacity for neuronal differentiation and migration (Reynolds & Weiss, 1992). Although there have been reports that transplantation of embryonic-derived stem cell progeny survive, only a limited number of

THir cells were identified in the graft (Svendsen et al., 1996) suggesting that this alternative is promising but not yet fully developed.

More recently, cell lines of immortalized tumor cells including a human embryonal carcinoma-derived neuronal population (hNT) have been developed. Transplantation of these hNT cells produced behavioural recovery from focal ischemia (Borlongan et al., 1998a; Borlongan et al., 1998b; Saporta et al., 1999) and quinolinic acid-induced striatal lesions (Hurlbert et al., 1999). hNT neurons have also been grafted into rats with experimental brain injury, however no significant improvement in behavioural recovery was noted (Muir et al., 1999; Philips et al., 1999). hNT neurons are derived from a human embryonic carcinoma cell line, NT2/D1 (Andrews, 1984). In contrast to other teratocarcinoma cell lines, which are capable of differentiating into neuronal, glial, and mesenchymal phenotypes, the NT2/D1 cells appear to be progenitor cells which have a progeny restricted to the neuronal lineage (called hNT neurons) following retinoic acid (RA) treatment (Andrews, 1984; 1987; Lee and Andrews, 1986; Abraham et al., 1991; Pleasure et al., 1992; Pleasure and Lee, 1993). The hNT neuronal progeny have been well characterized and it has been shown that these cells closely resemble human neurons (Pleasure et al., 1992; Pleasure and Lee, 1993). Furthermore, the hNT neurons bear glutamate receptor channels (Younkin et al., 1993), produce β-amyloid peptide (Mantione et al., 1995; Turner et al., 1996; Wertkin et al., 1993; Cook et al., 1997), and express mRNA for glutamic acid decarboxylase, choline acetyltransferase, and D_1 and D_2 DA receptors (Hurlbert et al., 1999). hNT neurons (Kleppner et al., 1995) or their precursor, NT2 cells (Miyazono et al., 1995; 1996), transplanted into the brains of immunodeficient nude mice survived for over 12 months without evidence of necrosis,

apoptosis, graft rejection, or tumor formation. Survival of hNT neurons transplanted into the cerebral cortex and hippocampus of cyclosporine-treated neonatal and adult Sprague-Dawley rats has also been demonstrated (Trojanowski et al., 1993). These grafts survived for up to 12 weeks and no tumor formation was observed. In a recent study, Konobu and colleagues observed that hNT neurons populated the photoreceptor layer as a stratum following epiretinal injections of the cells at 56 days and suggested that hNT neurons may take on the morphology and function of photoreceptors (Konobu et al., 1998).

The purpose of the present study was to determine whether hNT neurons survive when implanted into the striatum and substantia nigra (SN) of rats with unilateral 6-OHDA lesions of the dopaminergic nigrostriatal pathway and to assess the ability of these neurons to express TH and produce functional effects. We studied three different products of hNT neurons provided by Layton Bioscience, Inc. (Gilroy, CA). The products tested include hNT neurons and two hNT hybrids: hNT-DA neurons and lithium chloride (LiCl) pre-treated hNT-DA neurons. hNT neuron cultures were previously treated with RA for 6 weeks and then replated at one-third of the density in the presence of mitotic inhibitors, cytosine arabinoside, and fluorodeoxyuridine for 6 days. hNT-DA neuron are hNT neuron cultures treated with RA for only 4 weeks followed by replating and treatment with the same mitotic inhibitors. A shorter RA treatment time appears to enhance the number of cells expressing TH (personal communication, Mike McGrogan, Layton Bioscience, Inc.). The third product we used was LiCl pretreated hNT-DA neurons. These are hNT-DA neurons in which LiCl was added to the culture for 6 days during mitotic inhibitor treatment (personal communication, Mike McGrogan, Layton

Bioscience, Inc.). LiCl has been shown to promote the expression of TH in hNT neurons (Zigova et al., 1999).

Materials and methods

Study design

A total of 30 female Wistar rats (Charles River, St. Constant, Quebec, Canada) were used in this study. All animals received unilateral 6-OHDA lesions of the right nigrostriatal pathway and 27 rats later received intrastriatal and intranigral grafts (double grafts) of hNT neurons. Three hNT neuronal products (hNT neurons, hNT-DA neurons, LiCl pretreated hNT-DA neurons) were transplanted in this experiment. Sixteen animals received double grafts of hNT neurons, 7 received hNT-DA neurons, 4 received LiCl pretreated hNT-DA neurons and 3 served as controls and received a lesion only. Functional recovery was assessed by amphetamine-induced rotational behaviour.

Animals and 6-OHDA lesions

Twenty-seven female Wistar rats (Charles River) weighing 200 – 225 g, were housed 2 animals per cage with food and water *ad libitum* and allowed to acclimatize to the animal care facility for 7 days before surgery. All animal procedures were in accordance with the guidelines of the Canadian Council on Animal Care and the University Council on Laboratory Animals. Rats were anesthesized intramuscularly with 3.0 ml/kg of a ketamine-xylazine-acepromazine anesthetic mixture (25% ketamine hydrochloride; (Ketalean, MTC Pharmaceuticals, Cambridge, Ontario); 6% xylazine; (Rompun, Miles Canada, Etobicoke, Ontario); 2.5% acepromazine maleate; (Wyeth-

Ayerst Canada, Montreal, Quebec) in 0.9% saline and received two stereotactic injections of 6-OHDA (Sigma Chemical Company, Chicago, IL) (3.6 μg of 6-OHDA HBr/μl in 0.2 mg/ml of L-ascorbate in 0.9% saline) into the right ascending mesostriatal dopaminergic pathway at the following coordinates (mm): (1) 2.5 μl at anteroposterior (A/P): -4.0, mediolateral (M/L): -1.2, dorsoventral (D/V): -7.8, toothbar: -2.4; and (2) 3.0 μl of 6-OHDA at A/P: -4.0, M/L: -0.8, D/V: -8.0, toothbar: +3.4. The rate of injection was 1 μl / min with the cannula being left in place for 5 min before being slowly retracted. Animals were allowed to recover for 2 weeks in the animal care facility before being given an amphetamine challenge (5.0 mg / kg, ip) and their rotational scores were collected over a 70 min period using a computerized video activity monitor programmed for rotational behaviour (Videomex, Columbus Instruments, Columbus, Ohio). Only animals exhibiting a mean ipsilateral rotational score of eight or more complete full body turns / min were included in the study. Animals were tested for rotational behaviour at 3 and 6 weeks posttransplantation. Statistical analysis for between-group and within-group differences was assessed at $P<0.05$ using a two-way ANOVA followed by Tukey's *post hoc* test.

Preparation and transplantation of hNT cell suspensions

The frozen hNT neurons were obtained from Layton Bioscience (hNT neurons, hNT-DA neurons, and LiCl pretreated hNT-DA neurons) and stored at -80°C until the time of transplantation. Two weeks following 6-OHDA lesions, rats were chosen for transplantation if they exhibited a mean rotational score of eight full body turns per minute. Beginning on the day of surgery, each animal received 10 mg of cyclosporin A /

kg of body weight ip for the duration of the experiment. Prior to transplantation, the hNT neurons were quickly thawed by placing them in a water bath at 37°C. The cells were then washed three times in DMEM / 0.05% DNase (Sigma Chemical Company). The cells were suspended and the cell viability and suspension concentration calculated. The trypan blue dye exclusion method, which stains dead cells blue and fails to stain live cells, was used to assess cell viability (Table 1).

The cell suspensions were stereotactically injected both intrastriatally and intranigrally using a technique previously described (Mendez et al., 1996; Mendez and Hong, 1997). A specially designed capillary tip micropipette with an outer opening diameter of 50-70 μm is attached to a 2-μl Hamilton syringe and used to sterotatically implant the desired number of cells at a rate of 100 nl/min into both the SN and the striatum (400,000 cells / site). Each animal received a total of about 800,000 cells. Injection of the cells into the dorsolateral striatum occurs at the following coordinates (mm): (1) A/P: +1.3, M/L: -2.1, D/V: -5.5 and -4.3; (2) A/P: +0.6, M/L: -2.9, D/V: -5.5 and -4.3; and (3) A/P: +0.3, M/L: -3.7, D/V: -5.5 and -4.3; toothbar: -3.3; coordinates from Bregma and dorsal surface of the skull and the SN at the following coordinates (mm): 1) A/P: -4.8, M/L: -2.0, D/V: -8.3 and -8.1; 2) A/P: -5.0, M/L: -2.3, D/V: -8.2 and -8.0; and 3) A/P: -5.3, M/L: -2.6, D/V: -8.1 and -7.9; toothbar: -3.3; coordinates from Bregma and the dorsal surface of the skull.

Immunohistochemistry

At about 6 weeks, posttransplantation the rats were euthanized with an overdose of a ketamine-xylazine-acepromazine mixture and perfused transcardially with 200 ml of

0.1 M phosphate buffer (PB) followed by 250 ml of 4% paraformaldehyde in 0.1M PB for 10 min. The brains are then removed from the cranium to be postfixed with 4% paraformaldehyde in 0.1M PB, overnight before being stored for 24 h in phosphate-buffered saline (PBS) containing 30% sucrose. With the freezing microtome, 40-μm coronal sections were cut and stored in Millonig's solution (6% sodium azide in 0.1M PB) until immunohistochemical processing of the sections could be performed. Following processing sections were mounted in 0.1M PB on gelatin-coated slides and coverslipped with permount. Estimates of surviving cell numbers were calculated in every fourth section through the graft (6-10 sections per animal), using Abercrombie's formula (1946). The cell diameter used in the calculations for the Abercrombie's formula was 14μm, which was the average diameter measured of the THir cells. All data were analyzed for between-group and within-group differences at $P<0.05$ using a two-way ANOVA followed by Tukey's *post hoc* test.

Tyrosine hydroxylase

Staining for the presence of TH was performed using the primary rabbit anti-TH antibody (Ab;1:2500 Pel Freeze Biologicals, Rogers, AR) and the ABC-kit (Vector Laboratories Canada, Inc., Burlington, Ontario, Canada). For this procedure the sections were prewashed for 10 min in a solution of 10% methanol and 3% hydrogen peroxide (H_2O_2) and blocked in PB containing 0.3% Triton X-100 and 5% NSS for 1 h. The sections were removed and incubated in a 1:2500 solution of rabbit polyclonal anti-TH Ab for 16 h. To visualize Ab binding, 1:500 biotinylated swine anti-rabbit IgG Ab (Dako Diagnostics Canada, Inc., Mississauga, Ontario, Canada) is used followed by a

streptavidin-biotinylated horse radish peroxidase (HRP) complex kit. The peroxidase activity was visualized by the addition of 3,3'-diaminobenzidine (DAB) and 3% H_2O_2. The sections were then washed in 0.1M PB before being mounted.

Human neural cell adhesion molecule

Staining for the presence of neural cell adhesion molecule (NCAM) was performed using the primary mouse anti-human NCAM monoclonal antibody (Moc1; 1:1000 Dako Diagnostics Canada, Inc.) and the ABC kit. Briefly, the sections were prewashed for 30 min in a solution of 10% methanol and 3% hydrogen peroxide and blocked in PB containing 0.3% Triton X-100 and 5% normal horse serum for 1 h. The sections were removed and incubated in a 1:1000 solution of monoclonal mouse anti-NCAM (Moc1) Ab for 16 h. To visualize Ab binding, 1:250 biotinylated horse anti-mouse IgG Ab (Vector Laboratories Canada, Inc.) was used followed by a streptavidin-biotinylated HRP complex kit. The peroxidase activity was visualized by the addition of DAB and H_2O_2.

Human neuron-specific enolase

Staining for the presence of human neuron-specific enolase (NSE) was performed using the primary mouse anti-NSE monoclonal antibody (1:100; Vector Laboratories Canada, Inc.) and the ABC kit. The sections were prewashed for 30 min in a solution of 10% methanol and 3% hydrogen peroxide and blocked in PB containing 0.3% Triton X-100 and 5% NHS for 1 h. The sections were removed and incubated in a 1:100 solution of mouse monoclonal anti-NSE Ab for 16 h. To visualize Ab binding, 1:200 biotinylated

horse anti-mouse IgG Ab was used followed by a streptavidin-biotinylated HRP complex kit, DAB and H_2O_2.

Table 1. The mean (± SD) Viability of hNT Neurons and the number of animals transplanted with hNT, hNT-DA, and LiCl pretreated hNT-DA neurons

hNT neuronal product	Animals grafted	Viability	Total cells implanted
hNT	16	50.2 (±6.8)	~800,000
hNT-DA	7	47.8 (±9.1)	~800,000
LiCl pretreated hNT-DA	4	50.5 (±2.9)	~800,000

Results

Survival of hNT neuronal grafts

All animals that received both intrastriatal and intranigral grafts of the hNT neuronal products (Figure 1) had surviving grafts that were strongly immunostained for the presence of both human NSE (Figures 2A, 2B, 3A and 3B) and human NCAM (Figures 2C, 2D, 3C and 3D). Analysis of the hNT grafts by anti-NCAM immunohistochemistry (Figures 2C, 2D, 3C and 3D) revealed a strong staining of the entire graft area and darkly stained cell-like structures could clearly be seen within the graft boundary. The overall strong immunostaining of the graft made the determination of cell numbers impossible. NCAMir fibers extending beyond the graft-host interface could be seen in many of the grafted animals. NSE immunohistochemistry (Figures 2A, 2B, 3A and 3B) produced a similar strong staining pattern, with what appeared to be more darkly stained cells within the graft, but again counts could not be accurately determined. NSEir fibers were seen extending beyond the graft-host interface at the level of the striatum, and in some cases, fibers were observed to extend greater than 100 μm into the surrounding host tissue.

Expression of TH by hNT neurons

Analysis of TH expression in animals with hNT neuron grafts (n=16) showed no THir cells in either the striatum or the SN (Figures 2E and F). In 43% of animals with grafts of hNT-DA neurons (n=3), readily identifiable THir cells within both the striatum and the SN were observed. THir neurons appeared healthy and had processes extending for variable distances in the host brain. However, fiber outgrowth was sparse both within

the graft and in the host tissue surrounding the graft. In these animals, there were 435.12 ± 323.30 THir cells within the striatum and 393.68 ± 204.70 within the SN (Figure 5). THir cells were observed in 100% of animals with intrastriatal and intranigral grafts of LiCl pretreated hNT-DA neurons (Figure 4). The mean (± SD) number of THir cells within the intrastriatal and intranigral grafts was 489.39 ± 18.09 and 319.68 ± 142.08, respectively (Figure 5). There was no significant difference in the number of THir neurons between the hNT-DA neuronal and LiCl pre-treated hNT-DA neuronal grafts (P>0.05). Similarly, there was no significant difference in the number of THir cells between the intrastriatal and intranigral graft locations (P>0.05).

Amphetamine-induced rotational behaviour

There was not a statistically significant reduction in amphetamine-induced rotational behaviour at any of the time points tested regardless of the product implanted. In animals maintained for 6 weeks with double grafts of either hNT-DA neurons or LiCl pretreated hNT-DA neurons rotational behaviour exhibited a trend toward decreasing rotations, but this did not reach statistical significance. There was a correlation between surviving THir cells and rotational scores. Only animals that had surviving THir cells (43% of the hNT-DA group and the LiCl pretreated hNT-DA group) had decreased rotational scores while animals with no THir cells (hNT neuronal grafts and lesion only groups) did not exhibit any reduction in mean full body turns (Figure 6).

Figure 2.1. Representative parasagittal section through a double hNT grafted rat brain immunostained for human NSE (scale bar = 1000 μm).

Figure 2.1

Figure 2.2. Representative coronal sections through the level of the striatum and substantia nigra of rats with double grafts of hNT neurons immunostained for the presence of NSE (A and B). Adjacent sections were stained for the presence of NCAM (C and D) and TH (E and F). Although grafts are visualized following immunostaining for anti-NSE and -NCAM, note the absence of THir profiles in the grafted area on the lesioned side of the brain. (scale bar = 250 μm).

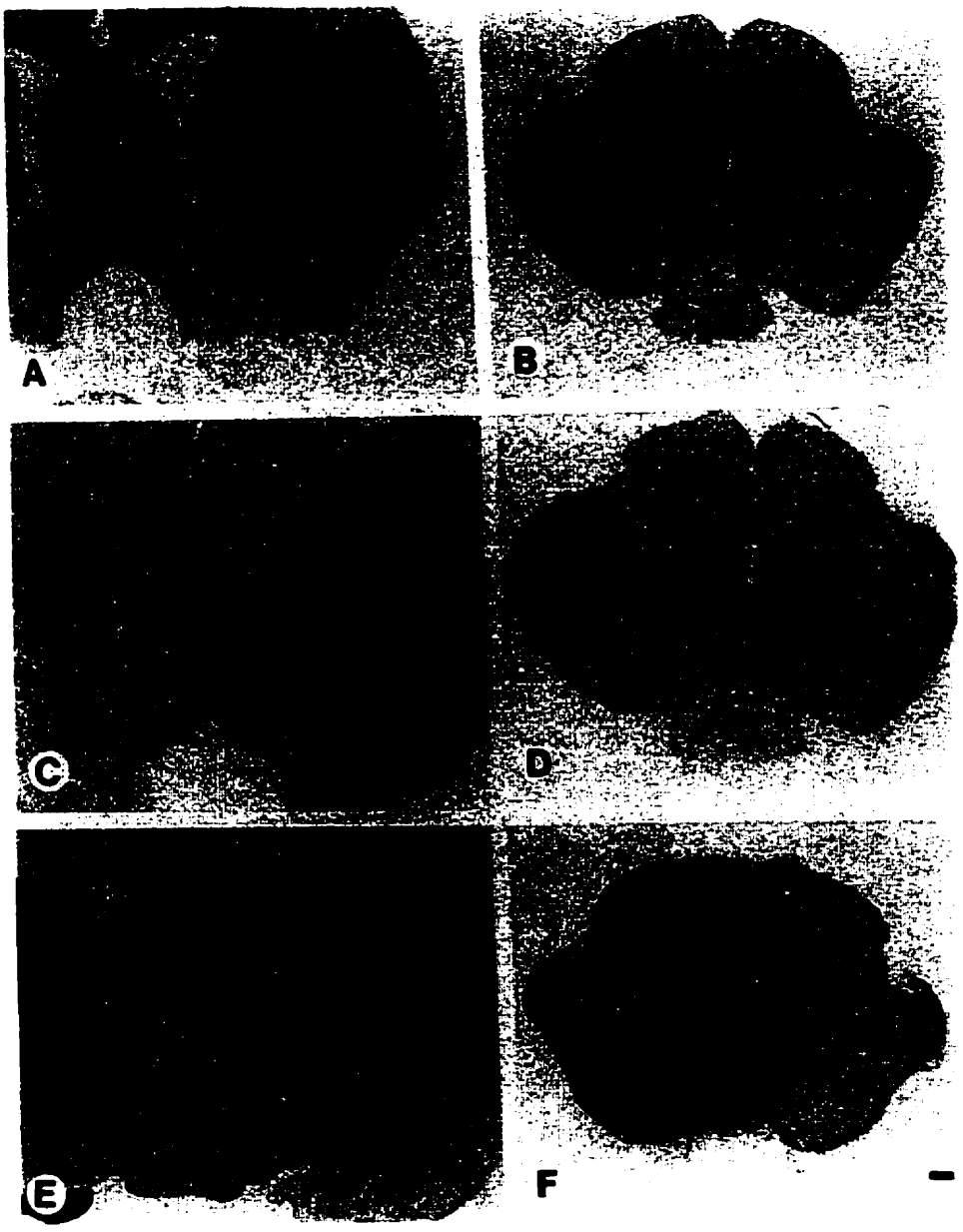

Figure 2.2

Figure 2.3. Higher power photomicrographs of intrastriatal and intranigral hNT neuronal grafts immunostained for human NSE (A and B) and NCAM (C and D). Note the dark staining of the graft that made counts of the number of surviving cells impossible (scale bar = 150 μm).

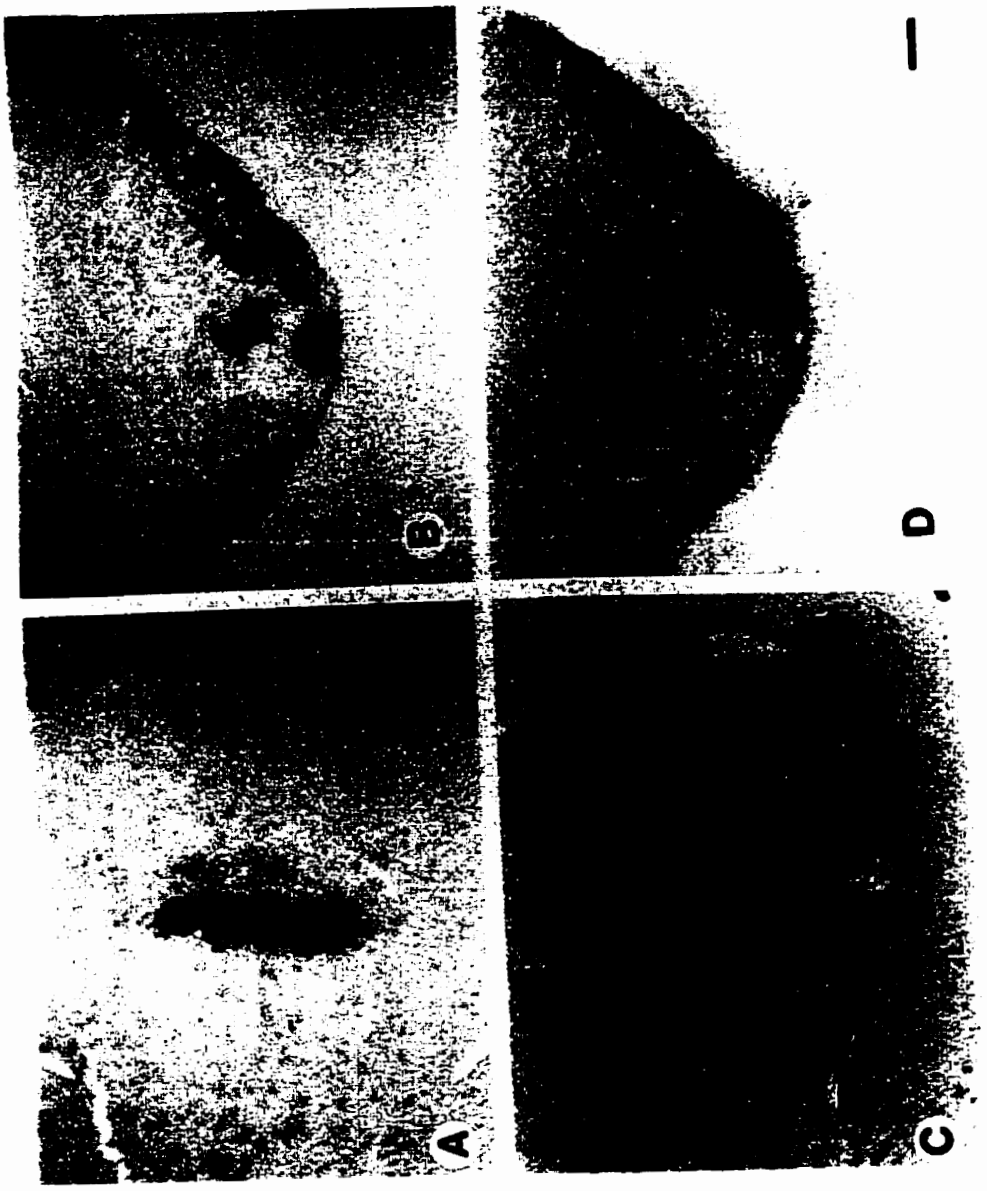

Figure 2.3

Figure 2.4. Intrastriatal (A-C) and intranigral (D-F) hNT-DA neuron grafts immunostained for NSE (A and D). Adjacent sections were immunostained for the presence of TH (B and E). C and F are higher power photomicrographs of B and E. Note that THir fibers can be seen extending from the cell bodies. (scale bar = A and D; 250 μm; B and E, 500 μm; C and F; 1000 μm).

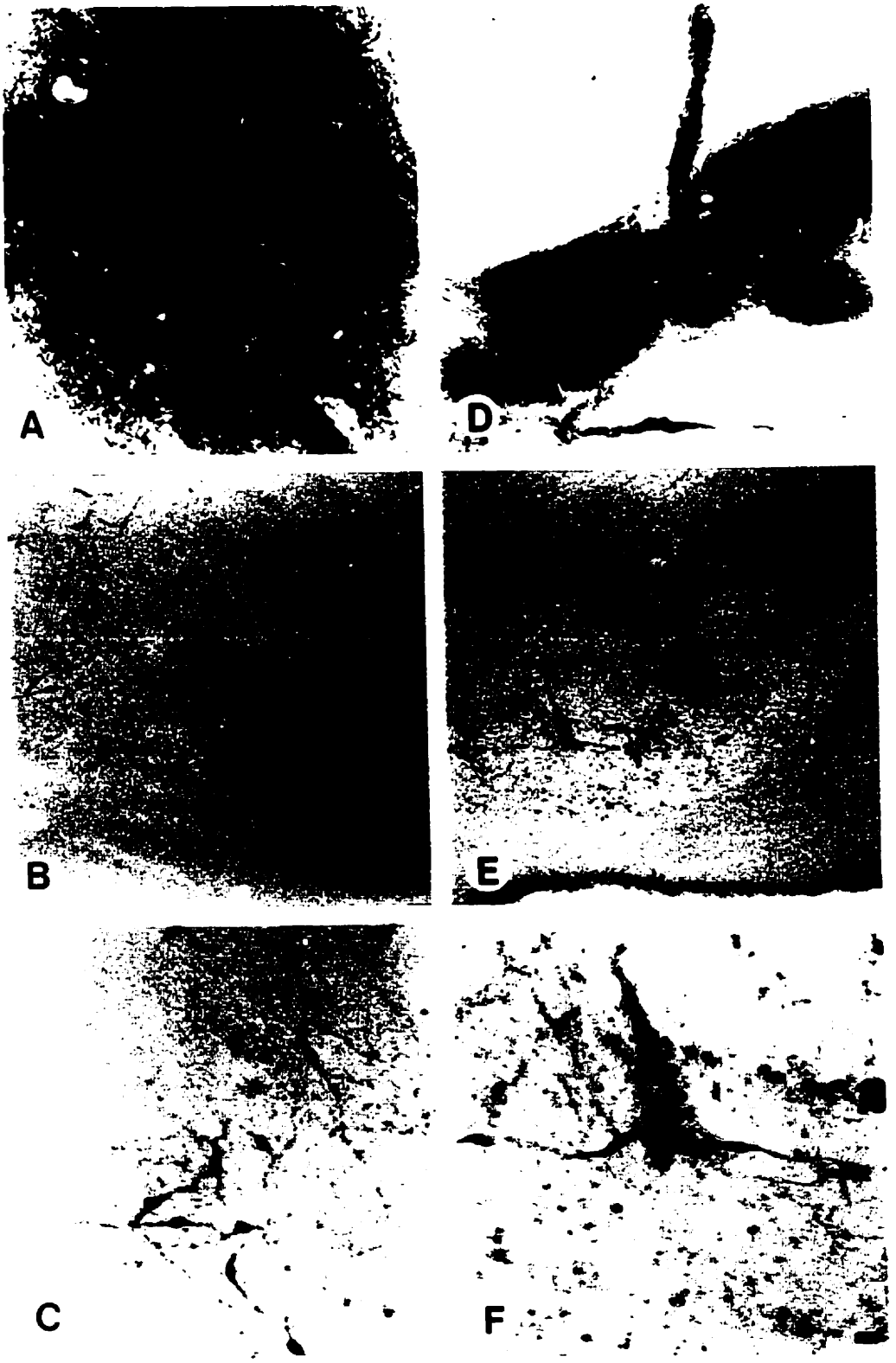

Figure 2.4

Figure 2.5. Bar graph demonstrating the mean (± SD) THir cells found within the intrastriatal and intranigral grafts of hNT neurons (white bars), hNT-DA neurons (stippled bars) and LiCl pretreated (gray bars) hNT-DA neurons. No significant difference in the number of cells was observed between the striatal or nigral location of the grafts. No surviving THir cells were encountered in animals grafted with hNT neurons. There was no significant difference in the number of THir neurons in rats grafted with hNT-DA neurons or LiCl pretreated hNT-DA neurons. However, only 43% of animals with hNT-DA neurons grafts contained THir neurons.

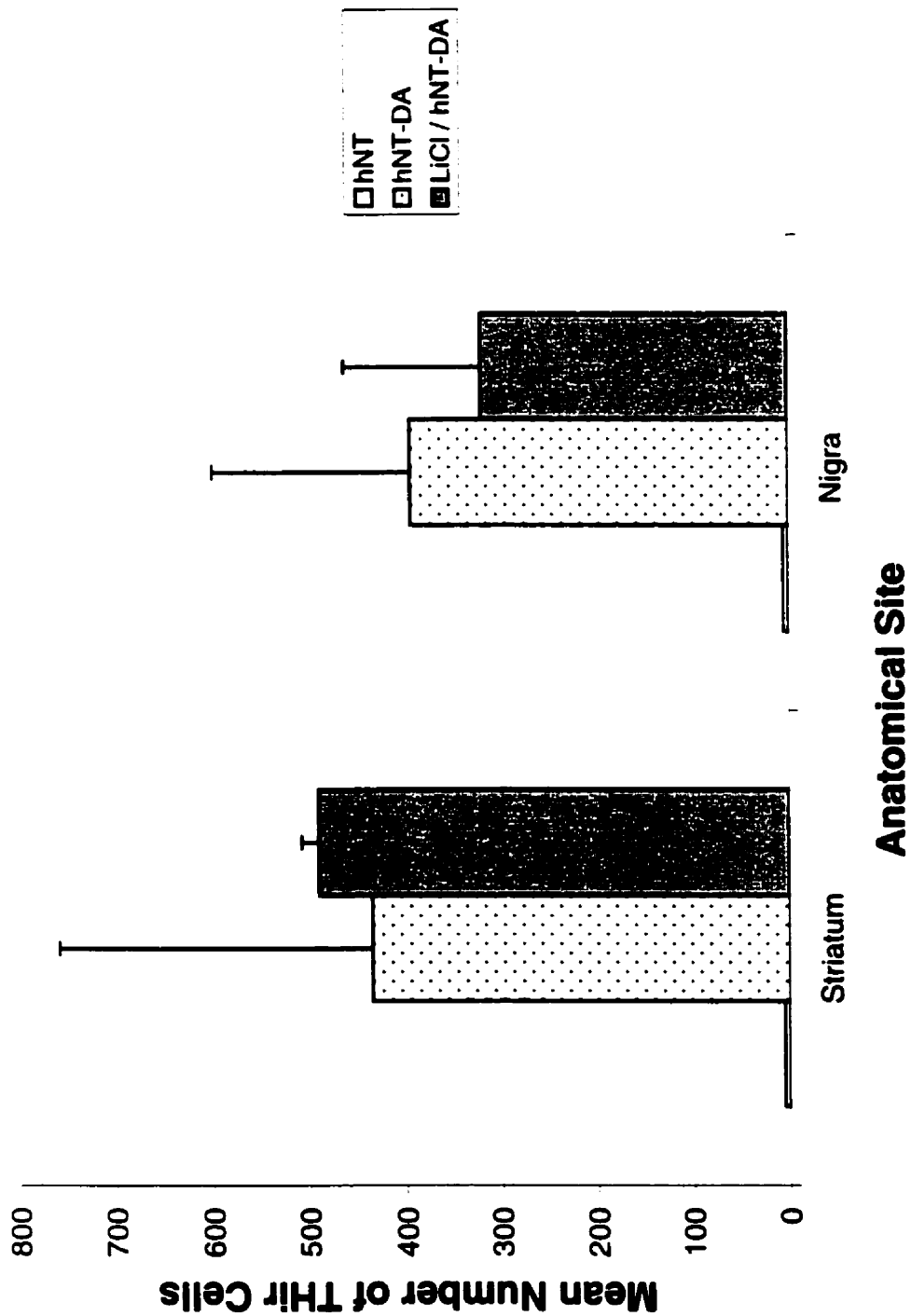
hNT
hNT-DA
LiCl / hNT-DA
Mean Number of THir Cells
800
700
600
500
400
300
200
100
0
Striatum
Nigra
Anatomical Site

Figure 2.5

Figure 2.6. The mean ± standard deviation (SD) rotations per minute with amphetamine challenge (5 mg/kg, ip), following 6-OHDA-induced lesions of the right ascending dopaminergic nigrostriatal pathway (Lesion) and 6 weeks following double grafting of medium only (white bars), hNT neurons (stippled bars), hNT-DA neruons (gray bars) and LiCl pretreated hNT-DA neurons (black bars). Although a reduction of rotational behaviour was observed in the hNT-DA neuron and LiCl pretreated hNT-DA neuron groups, this reduction did not achieve statistical significance.

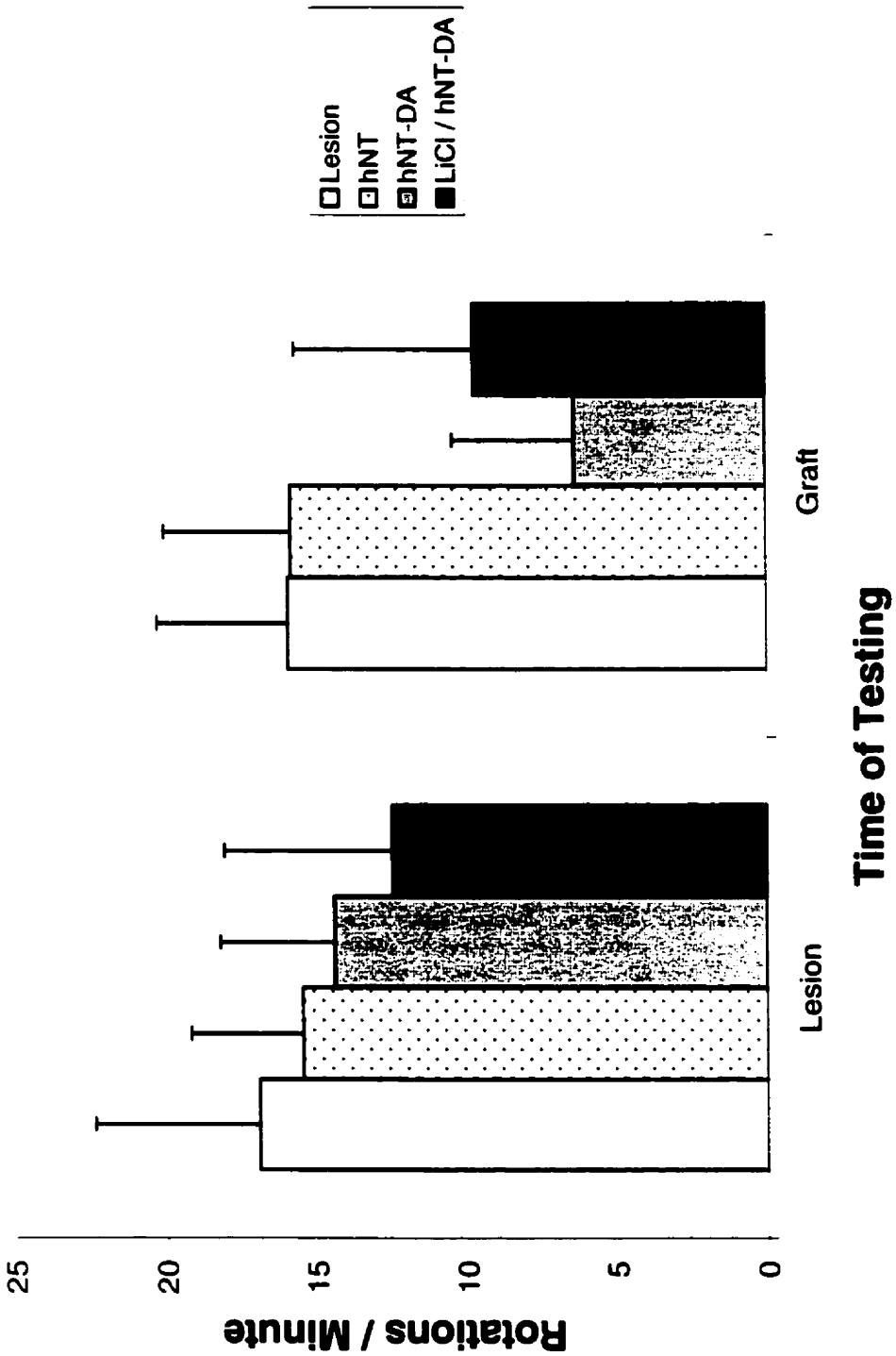
Lesion
hNT
hNT-DA
LiCl / hNT-DA
Rotations / Minute
25
20
15
10
5
0
Lesion
Graft
Time of Testing

Figure 2.6

Discussion

Graft survival

Immunostaining with anti-NCAM demonstrated that hNT neurons survive the transplantation procedure. This observation is in agreement with previous studies demonstrating survival of hNT neurons *in vivo* by using anti-NCAM immunohistochemistry (Trojanowski et al., 1993; Kleppner et al., 1995; Miyazono et al., 1995; 1996; Borlongan et al., 1998a; 1998b; Muir et al., 1999; Philips et al., 1999; Saporta et al., 1999). In addition, we have shown that hNT grafts can also be visualized with antibodies recognizing human NSE, which reflects the human origin of hNT neurons, originally derived from a human teratocarcinoma. TH immunohistochemistry of grafted hNT neurons demonstrated that the 6-week cultured hNT neurons fail to express TH, whereas in 43% of the animals receiving hNT-DA and 100% of animals with LiCl pretreated hNT-DA neuronal grafts there was evidence of THir neurons. The reason for TH expression in only 43% of animals receiving hNT-DA neuronal grafts is unknown but it is possible that these grafts may need time to mature and long-term studies are currently underway in our laboratory to address this issue. It is also possible that these cells need an additional factor such as LiCl to promote differentiation into a TH-expressing neuronal phenotype (Zigova et al., 1999). This concept is supported by the observation that 100% of animals grafted with hNT-DA neurons pretreated with LiCl had surviving THir neurons.

All of the animals with grafts of LiCl pretreated hNT-DA neurons exhibited THir cells within the grafts in the present study. A recent study reported that TH expression in hNT neurons was increased six-fold *in vitro* following 5 days of exposure to LiCl

(Zigova et al., 1999). Previous studies have also shown that *in vitro* lithium treatment increased the expression of TH in SH-SY5Y neuroblastoma cells (Chen et al., 1998) and bovine adrenal medullary cells (Terao et al., 1992). Other strategies to enhance the expression have also been used. Small increases in TH expression could be obtained when hNT neurons were cultured in the presence of acidic fibroblast growth factor, protein kinase pathway activators, and other coactivators (Iacovitti and Stull, 1997). Furthermore, Othberg and colleagues (1998) have also demonstrated a greater enhancement of TH expression by hNT neurons when cocultured with porcine Sertoli cells. Although these studies demonstrate an enhancement of TH expression in hNT neurons *in vitro*, it has yet to be determined whether these cells continue to express TH *in vivo*. There is also evidence that the hNT precursors, NT2 neurons, are capable of transfection with foreign genes (Trojanowski et al., 1997; Kofler et al., 1998) and hNT neurons are readily infected by vaccinia viruses (Cook et al., 1996), suggesting alternative methods for enhancing TH expression in these cells.

Interestingly, the number of surviving THir neurons was not different when transplanted in either the striatum or the SN. This suggests that the homotopic site (SN) environment does not influence the phenotype of hNT neurons. It has been reported that the mouse caudoputamen may influence the differentiation of hNT neurons into a dopaminergic phenotype (Miyazono et al., 1996), however, our study does not provide evidence that the rat striatum may influence the hNT neurons to differentiate into TH neurons to a greater extent when compared to the SN.

The failure of hNT neurons to provide functional recovery in the present study may relate to the relatively low number of THir neurons and poor fiber outgrowth

observed in surviving grafts. Fiber outgrowth and number of surviving THir neurons strongly correlate with the extent of functional recovery in fetal grafts (Rioux et al., 1991; Apostolides et al., 1998). It is possible that hNT cells mature at a slower pace than fetal dopaminergic neurons and long-term studies may be necessary to test this hypothesis.

hNT neurons as an alternative tissue source for neural transplantation?

The optimal cell for transplantation in Parkinsonian patients would be one that is not only abundant and readily available but also has the capability of synthesizing dopamine and reinnervating the nigrostriatal dopaminergic system. hNT neurons have some of these qualifications; they are readily available and able to proliferate in culture (Andrews, 1984; 1987; Lee and Andrews, 1986; Abraham et al., 1991; Pleasure et al., 1992; Pleasure and Lee, 1993). There is evidence that hNT neurons can survive transplantation into the adult rodent brain (Trojanowski et al., 1993; Kleppner et al., 1995). Reversal to their neoplastic phenotype has not been observed and the present study has shown that hNT neurons survive transplantation into the striatum and SN, integrate into the host, and express TH.

Although we have not shown that hNT neurons are capable of releasing dopamine, there is evidence that hNT neurons are immunopositive for markers of secretory activity *in vitro* (Pleasure et al., 1992). However, DA production may not be enough for functional restoration in PD. It is well known that grafting various cell lines transfected with the tyrosine hydroxylase gene reduces DA agonist-induced behavioural deficits in the Parkinson rat model (Wolff et al., 1989; Horellou et al., 1990a; 1990b; Fisher et al., 1991; Ishida et al., 1996; Lundberg et al., 1996; Tornatore et al., 1996;

Raymon et al., 1997; Fitoussi et al., 1998; Segovia et al., 1998). However, reinnervation of the host brain may also be crucial for restoring complex sensorimotor deficits in lesioned animals (Mendez et al., 1991; 1993; Rioux et al., 1991; Nikkhah et al., 1994; Mehta et al., 1998). hNT neurons may have the capability of producing and secreting dopamine and also reinnervating the host. This concept is supported by our observation that hNT neurons express TH after implantation and extend processes into the host brain. Further enhancement of host reinnervation could be accomplished by increasing the differentiation of hNT neurons into THir cells and promoting their fiber outgrowth. Our laboratory and several other investigators have demonstrated increased fiber outgrowth of dopaminergic transplants using GDNF (Rosenblad et al., 1996; Wang et al., 1996; Granholm et al., 1997; Apostolides et al., 1998; Mehta et al., 1998; Wilby et al., 1999) and BDNF (Yurek et al., 1996). It is possible that the addition of neurotrophic factors such as GDNF or BDNF to hNT neurons may similarly increase survival of THir neurons and induce fiber outgrowth.

Concluding remarks

This study has demonstrated that hNT neurons survive implantation, integrate into the host brain, and express TH when grafted into the striatum or SN. Although THir neurons were found in the striatum and SN, the numbers were relatively small and expression of a TH phenotype appeared to be independent of the site of implantation (striatum versus nigra). This study has also provided evidence that LiCl treatment may be beneficial in enhancing TH expression of hNT neurons.

hNT neurons are promising as a possible alternative to fetal tissue for transplantation in animal models of PD and may have potential clinical applications in the future. However, before hNT neurons can be considered a reliable cell source in experimental neural transplantation for PD, further improvements in enhancing TH expression are needed.

CHAPTER 3:

SIMULTANEOUS INTRASTRIATAL AND INTRANIGRAL DOPAMINERGIC GRAFTS IN THE PARKINSONIAN RAT MODEL: THE ROLE OF THE INTRANIGRAL GRAFT

The results presented in the following chapter have been submitted for publication in the Journal of Comparative Neurology.

Introduction

In the past decade, clinical trials of neural transplantation in which patients with PD have received intrastriatal fetal ventral mesencephalon (FVM) grafts have been conducted worldwide (Lindvall et al., 1989; 1990; 1992; 1994; Freed et al., 1992; Spencer et al., 1992; Widner et al., 1992; Peschanski et al., 1994; Freeman et al., 1995; Kordower et al., 1995; 1996; 1998; Wenning et al., 1997; Bluml, et al., 1999; Hagell et al., 1999; Hauser et al., 1999). Although the results reported in some transplanted patients are promising, clinical improvements have been limited and have not reached a level to justify the use of neural transplantation as a routine therapeutic procedure in PD. Although many variables contribute to the efficacy of neural transplantation in PD (Olanow et al., 1996; Mehta et al., 1997), optimal placement of the graft is likely a critical factor influencing the clinical outcomes of neural transplantation in PD.

To date, the main transplantation strategy in experimental and clinical PD has been to place dopaminergic grafts not in their ontogenic site (SN) but in their target area (striatum) (Björklund et al., 1980; 1983; Dunnett et al., 1983; Lindvall et al., 1989; Mendez et al., 1991; Freed et al., 1992; Widner et al., 1992; Freeman et al., 1995). Although intrastriatal dopaminergic grafts are capable of reinnervating the striatum, they fail to restore the nigrostriatal circuitry (Doucet et al., 1989; Mendez et al., 1991). Furthermore, dendritic DAergic control of SNr activity, which is important in the regulation of basal ganglia outflow (Cheramy et al., 1979; 1981; Gauchy et al., 1987; Robertson, 1992a), can not be achieved by intrastriatal grafts alone. Dopaminergic reinnervation of other nigral targets such as the STN and globus pallidus GP is also lacking. Recent evidence in unilaterally

6-OHDA-lesioned rats has shown that upregulation of cytochrome oxidase and *c-fos* gene expression in the STN and GP is not normalized by intrastriatal grafts (Nakao et al., 1998). The failure to restore basal ganglia circuitry by ectopically placed intrastriatal grafts may be an important factor limiting the efficacy of fetal tissue transplantation in Parkinsonian patients.

We have hypothesized that simultaneous intrastriatal and intranigral dopaminergic grafts (double grafts) may provide a more complete restoration of the nigrostriatal circuitry. This hypothesis is supported by the demonstration that double grafts promote some degree of reconstruction of the nigrostriatal pathway and a quicker and more complete rotational recovery in the rodent model of PD (Mendez et al., 1996; Mendez and Hong, 1997). Reinnervation of both the striatum and SN may be essential to optimize graft-derived functional improvement. We postulate that double grafts may be a superior strategy in neural transplantation for PD. This notion is further supported by a recent study in which enhanced recovery was observed in hemiparkinsonian rats with simultaneous intrastriatal dopaminergic and intranigral GABAergic grafts (Winkler et al., 1999).

The present study was designed to investigate the role of the intranigral DAergic graft in restoring function in 6-OHDA-lesioned rats transplanted with simultaneous intrastriatal and intranigral grafts. The results of this study showed that the functional recovery, achieved by rats implanted with double grafts was reversed by the subsequent destruction of the intranigral graft. This observation strongly suggests that restoration of the dopaminergic input to the SN by the intranigral graft is crucial for the functional recovery observed in double grafted animals.

Materials and methods

Experimental design

A total of 20 female Wistar rats (Charles River, St. Constant, Quebec), weighing between 200 – 250 g, housed two animals per cage with food and water *ad libitum* were used for this experiment. Unilateral 6-OHDA lesions of the right ascending nigrostriatal pathway were performed in all of the animals (see *First 6-OHDA Lesion* below). Ten animals received single intrastriatal grafts of FVM cells. The intrastriatally-grafted animals were subdivided in 2 groups and received either a second 6-OHDA (STR-6OH) or vehicle (STR-VEH) injection in the SN 10 weeks after transplantation (Figure 1). Ten animals received double grafts of FVM cells in both the striatum and SN. The double grafted animals were also subdivided equally in 2 groups. One group (DBL-6OH) received a second 6-OHDA lesion in the region of the intranigral graft and the second group (DBL-VEH) received an injection of vehicle in the same region 10 weeks after transplantation (Figure 1). The time course of this study, from the day the animals arrived, until their brains were processed for TH immunohistochemistry, is shown in Figure 2. All animal procedures were in accordance with the guidelines of the Canadian Council on Animal Care and the University Council on Laboratory Animals.

First 6-OHDA lesion

Rats were anesthesized, intramuscularly with 3.0 ml / kg of a ketamine-xylazine-acepromazine anesthetic mixture (25% ketamine hydrochloride; Ketalean, MTC Pharmaceuticals; 6% xylazine; Rompun, Miles Canada; 2.5% acepromazine maleate;

Wyeth-Ayerst Canada; in 0.9% saline) and received two stereotactic injections of 6-OHDA (Sigma Chemical Company) (3.6 μg of 6-OHDA HBr/μl in 0.2 mg/ml of L-ascorbate in 0.9% saline) into the right ascending nigrostriatal DAergic pathway. 6-OHDA injections occurred at the following coordinates (mm): (1) 2.5 μl at A/P: -4.0, M/L: -1.2, D/V: -7.8, toothbar: -2.4; and (2) 3.0 μl of 6-OHDA at A/P: -4.0, M/L: -0.8, D/V: -8.0, toothbar: +3.4. The rate of injection was 1 μl / min. and the cannula was left in place for 5 min before slowly being retracted. Animals were allowed to recover for 2 weeks in the animal care facility before being given an amphetamine challenge (5.0 mg/kg, ip). Their rotational scores were collected over a 70-minute period using a computerized video activity monitor programmed for measuring rotational behaviour (Videomex V, Columbus Instruments). Only animals exhibiting a mean ipsilateral rotational score of eight or more complete full body turns per minute were included in the study.

Second 6-OHDA lesion

Nine to ten weeks following transplantation, grafted animals received a second injection of 3.6 μg of 6-OHDA HBr / μl in 0.2 mg/ml of L-ascorbate in 0.9% saline (0.9 μl / site) or vehicle (2 mg/ml ascorbic acid / 0.9% saline) (0.9 μl / site) at the same co-ordinates in which the intranigral graft was placed (see *Transplantation* below). All injections of vehicle or 6-OHDA were performed at the following coordinates (mm): 1) A/P: -4.8, M/L: -2.0, D/V: -8.3 and -8.1; 2) A/P: -5.0, M/L: -2.3, D/V: -8.2 and -8.0; and 3) A/P: -5.3, M/L: -2.6, D/V: -8.1 and -7.9; toothbar: -3.3; coordinates from Bregma and the dorsal surface of the skull at Bregma.

Rotational behaviour

Two weeks after the first 6-OHDA lesion, and every three weeks following transplantation and the second 6-OHDA injection, rats were challenged with amphetamine (5 mg/kg; ip) (Figure 2). Rotational behaviour was analyzed for 70 minutes following amphetamine injection, using a computerized-video activity monitor system (Videomex, Columbus Instruments).

Transplantation

Thirteen to fourteen day old rat fetuses were removed from pregnant female rats under sodium pentobarbital anesthesis. Ventral mesencephalic tissue was harvested under sterile conditions. The FVM tissue was washed 3 times in 0.05% DNase / DMEM (DNase and DMEM: Sigma Chemical Company), placed for 20 mintues at 37°C in DNase / DMEM / 0.1% trypsin (trypsin: Sigma Chemical Company) and then rinsed 4 times with 0.05% DNase / DMEM. The tissue was then mechanically dissociated until a milky, homogeneous single-cell suspension was achieved. The Trypan Blue dye exclusion method was used to assess cell viability and cell suspension concentration (Table 1).

The cell suspensions were stereotactically injected into either the striatum alone or both the striatum and SN incorporating the transplantation technique previously described (Mendez et al., 1996; Mendez and Hong, 1997; Apostolides et al., 1998). A specially designed capillary tip micropipette with an outer opening diameter of 50-70 μm is attached to a 2-μl Hamilton syringe and used to stereotatically implant the cell suspension at a rate of 100 nl/min. The single grafted rats (STR-VEH, STR-6OH)

received 400,000 cells in the striatum and an equal volume of medium in the SN. The double grafted animals received 400,000 cells in the striatum and 400,000 cells in the SN for a total of 800,000 cells (Table 1). Injection of the cells into the dorsolateral striatum occured at the following coordinates (mm): 1) A/P: +1.3, M/L: -2.1, D/V: -5.5 and -4.3; 2) A/P: +0.6, M/L: -2.9, D/V: -5.5 and -4.3; and 3) A/P: +0.3, M/L: -3.7, D/V: -5.5 and -4.3; toothbar: -3.3; and the SN at the following coordinates (mm): 1) A/P: -4.8, M/L: -2.0, D/V: -8.3 and -8.1; 2) A/P: -5.0, M/L = -2.3, D/V = -8.2 and -8.0; and 3) A/P: -5.3, M/L: -2.6, D/V: -8.1 and -7.9; toothbar: -3.3; coordinates from Bregma and the dorsal surface of the skull at Bregma.

Tyrosine hydroxylase immunohistochemistry

Staining for the presence of TH was performed using the primary rabbit anti-TH antibody (1:2500 Pel Freeze Biologicals) and the ABC-kit (Vector Laboratories Canada, Inc.). For this procedure, sections were prewashed for 10 min in a solution of 10% methanol and 3% hydrogen peroxide and blocked in PB containing 0.3% Triton X-100 and 5% normal swine serum for 1 h. The sections were removed and incubated in a 1:2500 solution of rabbit polyclonal anti-TH antibody for 16 h. To visualize antibody binding, 1:500 biotinylated swine anti-rabbit IgG antibody (Dako Diagnostics Canada, Inc.) was used followed by a streptavidin-biotinylated HRP complex kit followed by the addition of DAB and H_2O_2. The sections were then washed in 0.1M PB, placed on gelatinous slides and dehydrated before mounting and coverslipping in permount.

Cell counts and statistical analysis

The total number of surviving THir cells was estimated using Abercrombie's formula (Abercrombie, 1946). The mean diameter was calculated for thirty cells selected randomly within each experimental group. Cells were randomly selected by their location within a 0.1 X 0.1 mm ocular grid placed over the graft. The diameters were then calculated using a computer system equipped with Optimas image analysis software (Optimas Corporation, Bothell, WA). The mean cell diameter was calculated for each experimental group and substituted into Abercrombie's equation. Sixteen to twenty sections were counted in each animal.

Within and between group differences for amphetamine-induced rotational behaviour was performed at $P<0.05$ using a two-way ANOVA followed by Tukey's *post hoc* test. Between group differences for THir cell survival were calculated at $P<0.05$ using a Student's paired

T-test.

Figure 3.1. Schematic representation of the experimental groups involved in this study. 20 rats were used, and received either intrastriatal (n=10) or double DAergic grafts (n=10). These groups were further subdivided and received either intranigral vehicle (STR-VEH; DBL-VEH) or 6-OHDA injections (STR-6OH; DBL-6OH).

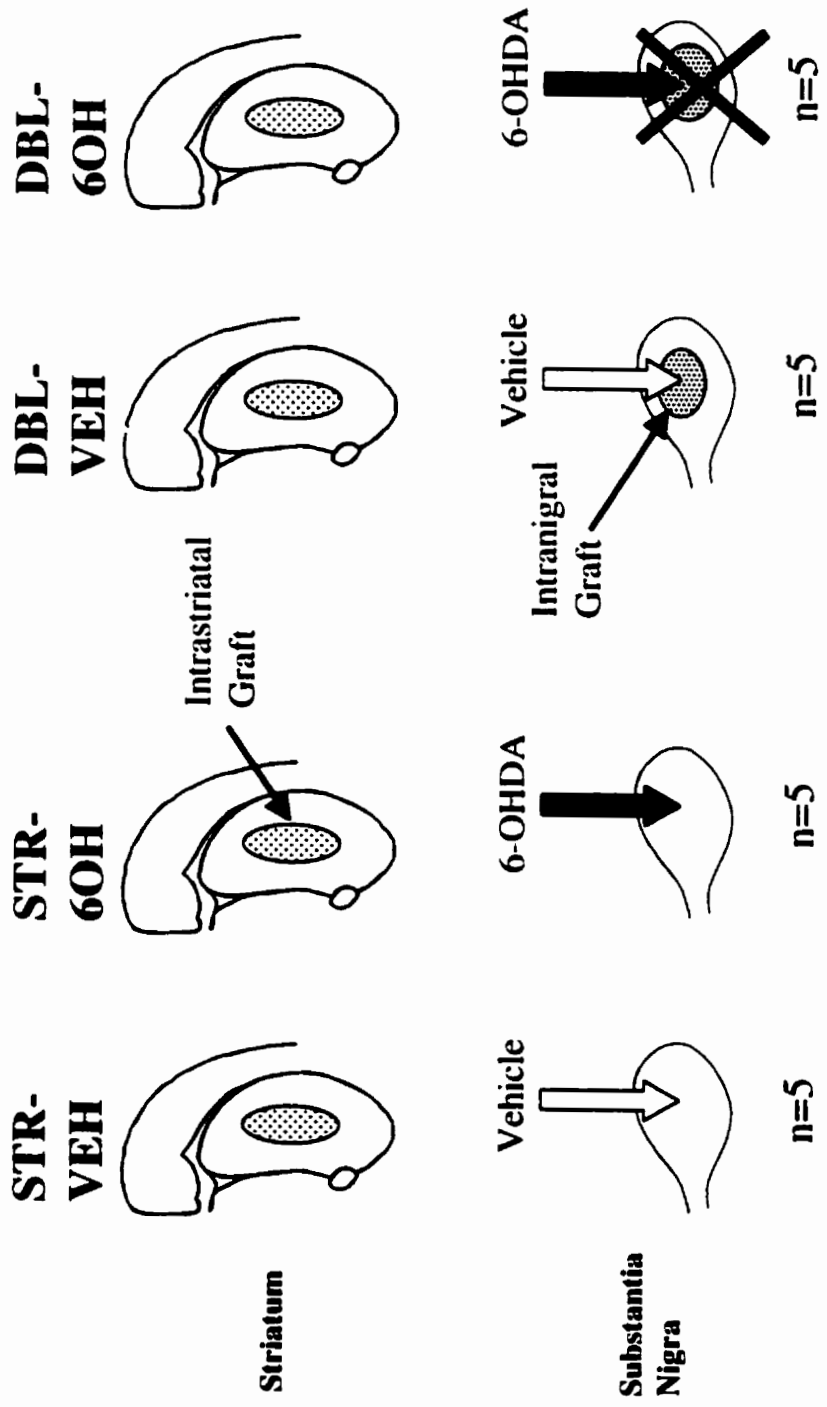
STR-
VEH
STR-
6OH
DBL-
VEH
DBL-
6OH
Striatum
Intrastriatal
Graft
Vehicle
6-OHDA
6-OHDA
Substantia
Nigra
Intranigral
Graft
Vehicle
n=5
n=5
n=5
n=5

Figure 3.1

Figure 3.2. Time-line representing the sequence of procedures conducted during the duration of this experiment.

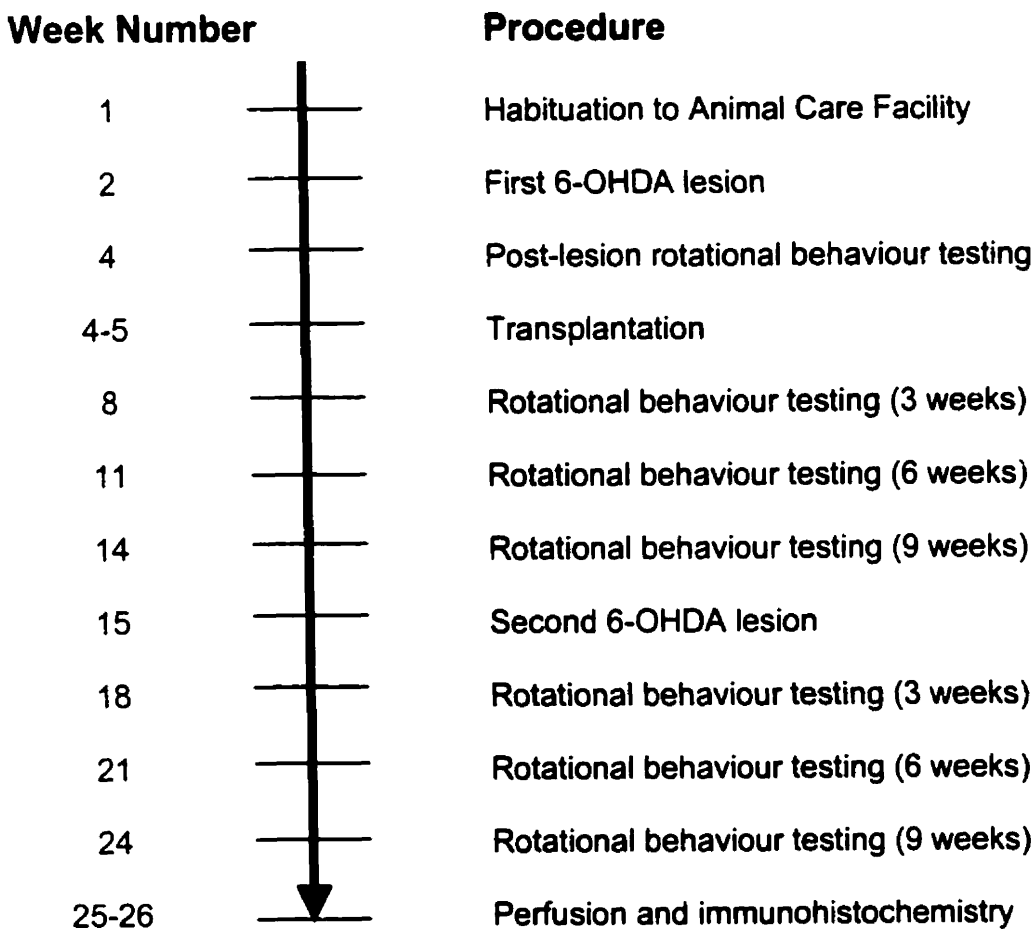
Week Number
Procedure
1
Habituation to Animal Care Facility
2
First 6-OHDA lesion
4
Post-lesion rotational behaviour testing
4-5
Transplantation
8
Rotational behaviour testing (3 weeks)
11
Rotational behaviour testing (6 weeks)
14
Rotational behaviour testing (9 weeks)
15
Second 6-OHDA lesion
18
Rotational behaviour testing (3 weeks)
21
Rotational behaviour testing (6 weeks)
24
Rotational behaviour testing (9 weeks)
25-26
Perfusion and immunohistochemistry

Figure 3.2

Table 2. Details of the total cell number and viability transplanted in each group.

Group	Cell Viability (%; ± SD)	Total Cells Implanted
STR-VEH	95.14 ± 4.41	~ 400,000 (STR)
STR-6OH	99.10 ± 0.45	~ 400,000 (STR)
DBL-VEH	95.00 ± 4.24	~ 800,000 (STR + SN)
DBL-6OH	94.38 ± 4.63	~ 800,000 (STR + SN)

STR = striatum SN = substantia nigra

Results

Effect of the First 6-OHDA Lesion

Injections of 6-OHDA within the nigrostriatal pathway resulted in a virtually complete absence of THir cell bodies and fibers within the ipsilateral SN, medial forebrain bundle and fibers within the ipsilateral striatum. 6-OHDA-lesioned animals exhibited a strong clockwise circling behaviour when challenged with amphetamine 2 weeks following the lesion (Figure 3).

Transplants

a) Double grafts

In double grafted animals with a subsequent vehicle injection (DBL-VEH), robust surviving grafts were observed within the striatum and nigra (Figures 4; 5A, 5B; 6A, 6B). These grafts were marked by the presence of many surviving THir cell profiles and fibers within the graft. THir fibers were also seen extending beyond the boundary of the intrastriatal graft, reinnervating the host striatum. In those animals, THir fibers presumably originating from within the intrastriatal graft, extended caudally into the GP and internal capsule along a trajectory towards the intranigral graft (Figure 4B).

In DBL-6OH animals, robust grafts were observed in the striatum alone. Numerous THir cell bodies and fibers were observed within the graft as well as good fiber outgrowth into the host striatum (Figure 5C). However, the grafts in the SN were very small (Figures 5D; 6C, 6D). Many of the remaining THir cells were dystrophic with much shorter THir fibers compared to the intranigral grafts of DBL-VEH animals (Figures 5B, 5D).

b) Single grafts

Animals with intrastriatal grafts (STR-VEH, STR-6OH) had healthy grafts with numerous cell bodies and fibers within the graft and robust fiber outgrowth into the surrounding host striatum. No THir cells were encountered in the SN in the group of animals (STR-6OH) receiving a second 6-OHDA lesion in the nigral area (Figure 6E, 6F).

c) Cell counts

The mean (±SD) number of THir cells within the intrastriatal graft in the 4 groups were: STR-VEH = 1077.17 ± 500.39; STR-6OH = 1290.12 ± 409.40; DBL-VEH = 1054.26 ± 254.49 and DBL-6OH = 1402.93 ± 635.25. There was no significant difference in the number of cells within the intrastriatal graft in any of the groups (Figure 7). The mean (±SD) number of THir cells within the nigral region in the 4 groups were: STR-VEH and STR-6OH = 0; DBL-VEH = 915.33 ± 244.94 and DBL-6OH = 267.69 ± 68.41. A significantly fewer number of surviving THir cells ($P<0.01$) within the intranigral graft was observed in the DBL-6OH group when compared to the DBL-VEH group (Figure 7).

Behavioural recovery

a) Post-transplantation behavioural recovery

Nine weeks following transplantation there was a dramatic reduction in amphetamine-induced rotational behaviour in all groups ($P<0.0002$) (Figure 3). The level of recovery did not differ significantly among the groups.

b) Effect of the second lesion

At 9 weeks after the second lesion, there was a significant elevation ($P<0.001$) in the number of rotations in the DBL-6OH group when compared to all other groups (Figure 3). This elevation of rotational scores was also significant when compared to the scores of the same group of animals obtained 9 weeks after transplantation. This reversal in rotational recovery was observed at the earliest time-point, 3 weeks following the 2nd lesion and was sustained for the duration of the study. No significant change in rotational behaviour was observed in the other groups when compared to each other or their 9-week post-grafting values.

Figure 3.3. Graph demonstrating the mean (± SD) amphetamine-induced full body turns / minute for each group, 2 weeks following the initial 6-OHDA lesion (Lesion), 9 weeks following transplantation (Graft) and 9 weeks following the second lesion (2nd Lesion). *P<0.0002, compared to rotational scores after the first 6-OHDA lesion; **P<0.0005, compared to rotational scores for all the groups 9 weeks post-grafting; ***P<0.001, compared to all the groups following the 2nd lesion. STR-VEH = intrastriatally grafted animals with a second vehicle injection; STR-6OH = intrastriatally grafted animals with a second 6-OHDA lesion; DBL–VEH = double grafted animals with a second vehicle injection; DBL-6OH = double grafted animals with a second 6-OHDA lesion.

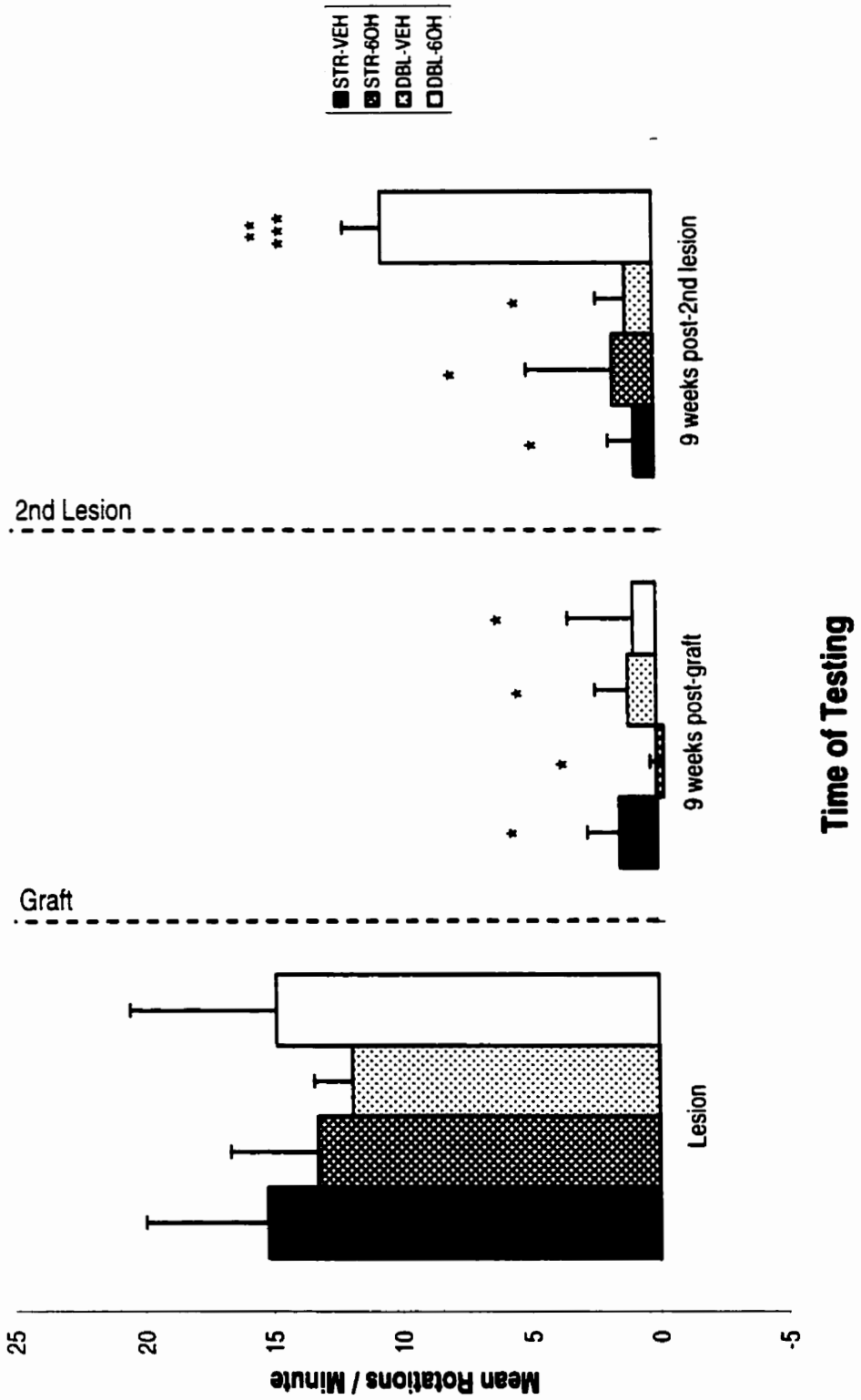
STR-VEH
STR-6OH
DBL-VEH
DBL-6OH
Mean Rotations / Minute
25
20
15
10
5
0
-5
Lesion
Graft
9 weeks post-graft
2nd Lesion
9 weeks post-2nd lesion
Time of Testing

Figure 3.3

Figure 3.4 – Representative parasagittal section through a double grafted rat brain demonstrating robust survival of intrastriatal and intranigral FVM grafts (A). Note the halo of dense THir surrounding the intrastriatal graft. (B) and (C) are higher power photomicrographs of the intrastriatal and intranigral grafts (B, C). In (B), note the THir fibers, most likely from the intrastriatal graft, extending into the globus pallidus. Scale bar: A = 500 μm; B and C = 50 μm.

Figure 3.4

Figure 3.5 – Representative coronal sections through double grafted rats (DBL-VEH; DBL-6OH) at the levels of the intrastriatal and intranigral grafts. Many surviving THir cells and fibers can be observed within both intrastriatal grafts (A, C). A robust intranigral graft can be seen in the DBL-VEH animal (B). A very small intranigral graft can be seen after a second 6-OHDA lesion in a DBL-6OH animal (D). Scale bar = 100 μm.

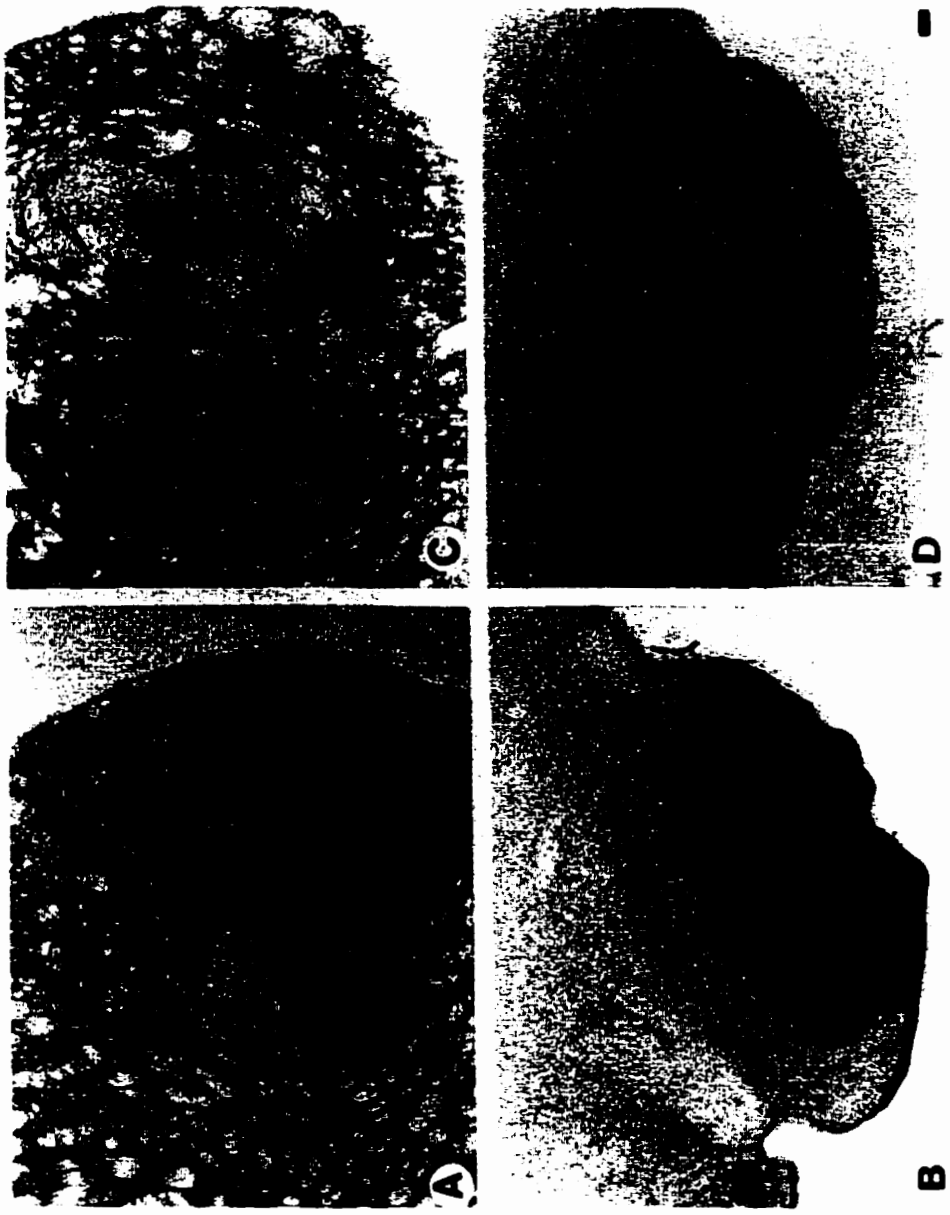

Figure 3.5

Figure 3.6 – Representative coronal sections at the level of the substantia nigra of DBL-VEH (A, B), DBL-6OH (C, D) and STR-6OH animals (E, F). A robust intranigral graft is seen in a rat with a subsequent intranigral vehicle injection (A, B). In contrast, double grafted animals with subsequent 6-OHDA injections (DBL-6OH) had very small grafts (C, D). Animals with intrastriatal grafts and subsequent 6-OHDA injections (STR-6OH) had no surviving THir cells in the substantia nigra (E, F) Scale bar: A, C, E = 500 μm and B, D, F = 200 μm.

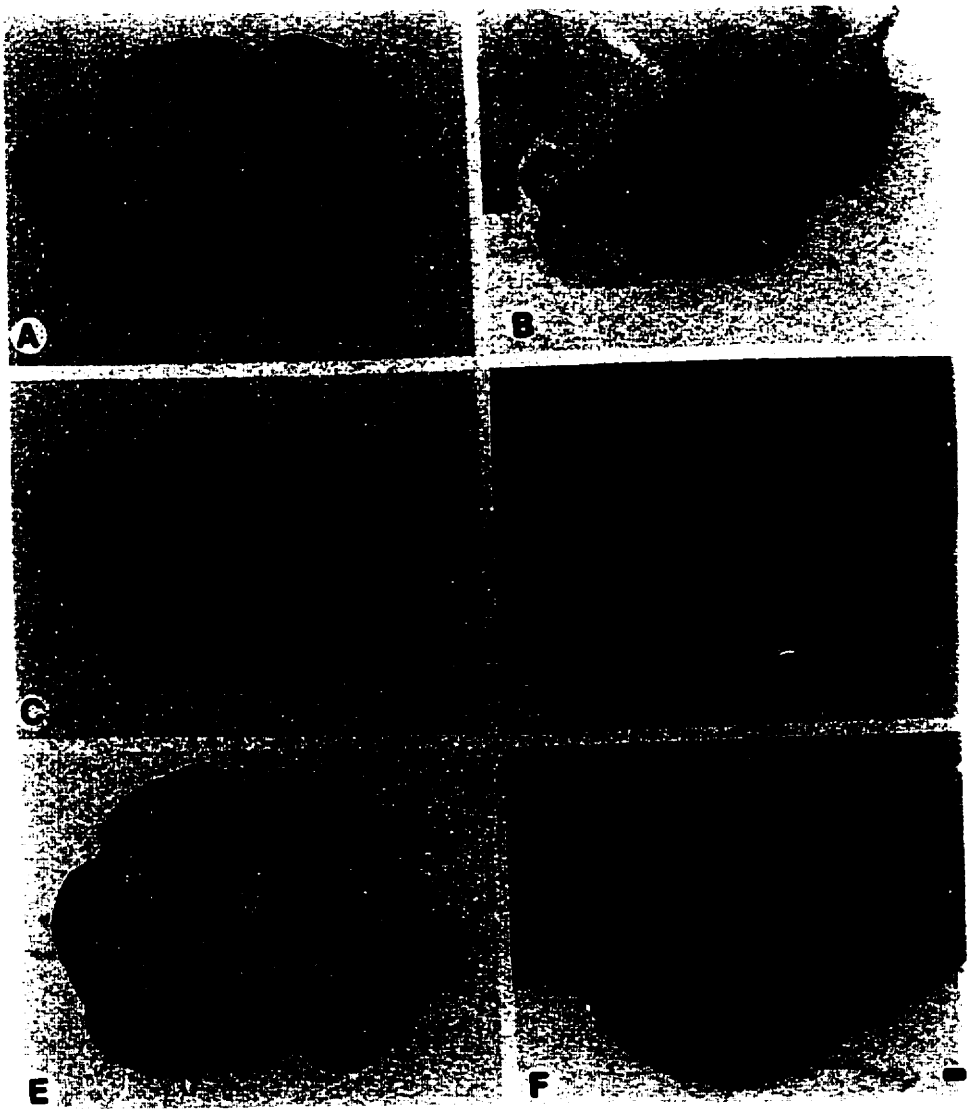

Figure 3.6

Figure 3.7 – Graph depicting the mean (±SD) surviving number of THir cells within the intrastriatal and intranigral grafts. Significantly fewer cells were observed in the intranigral graft of double grafted animals with a subsequent 6-OHDA injection (DBL-6OH) as compared to animals with a subsequent vehicle injection (DBL-VEH) (*P<0.001).

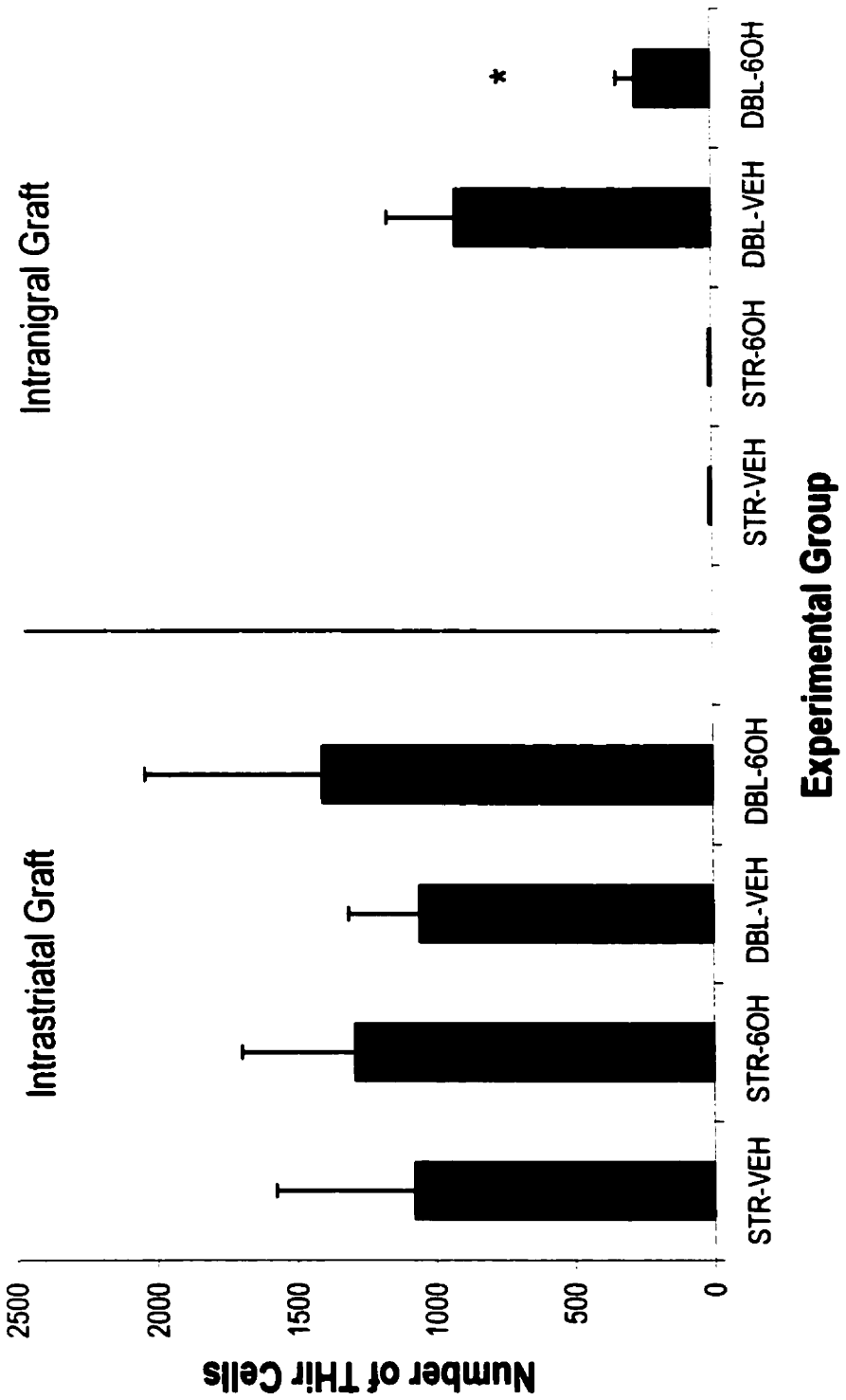
Intrastriatal Graft
Intranigral Graft
Number of THir Cells
2500
2000
1500
1000
500
0
*
STR-VEH
STR-6OH
DBL-VEH
DBL-6OH
STR-VEH
STR-6OH
DBL-VEH
DBL-6OH
Experimental Group

Figure 3.7

Discussion

In the current neural transplantation strategy for PD, the striatum has been targeted as the optimal site for DAergic graft placement (Björklund et al., 1980; 1983; Dunnett et al., 1983; Lindvall et al., 1989; Mendez et al., 1991; Freed et al., 1992; Widner et al., 1992; Freeman et al., 1995). The main reason for this ectopic placement of DAergic tissue is the apparent inability of grafts placed in the ontogenic location (SN) to grow axons over long distances to reach their target (striatum) (Björklund et al., 1983; Dunnett et al., 1989; Nikkhah et al., 1994a). However, this strategy has failed to restore dopaminergic innervation to the SN or reconstruct the nigrostriatal pathway. The inability of intrastriatal grafts to restore the dopaminergic nigrostriatal circuitry may be an important factor limiting the clinical efficacy of fetal transplantation in parkinsonian patients. We have previously demonstrated that simultaneous nigral grafts placed in both the striatum and the SN (double grafts) induce a faster and more significant reduction in rotational behaviour upon amphetamine challenge when compared to intrastriatal grafts alone (Mendez et al., 1996). This beneficial effect could be partially attributed to an increase in striatal reinnervation (Mendez and Hong, 1997) but may also result from restoration of DAergic reinnervation to the host SN.

In the present study we have demonstrated that the intranigral graft is important in the behavioural recovery of rats receiving simultaneous intrastriatal and intranigral grafts. Double grafted rats that received a second 6-OHDA injection in the region of the intranigral graft exhibited a reversal of the rotational recovery achieved after transplantation. This change in rotational behaviour correlated well with damage of the intranigral graft by the toxin. Animals that received vehicle injections in the region of the

intranigral graft had no reversal in the functional recovery gained after transplantation. The reversal of rotational recovery can not be explained by possible damage to the intrastriatal graft by the second 6-OHDA injection because all groups had healthy grafts with no significant difference in the number of surviving THir neurons. Furthermore, the increase in amphetamine-induced rotations appears to be directly attributed to the destruction of the transplanted intranigral FVM cells and not to destruction of residual host nigral DAergic cells that may have escaped the first lesion. This concept is strongly supported by the observation that no detrimental effect in rotational behaviour was detected in intrastriatally grafted animals that received subsequent intranigral 6-OHDA injections.

It is well known that dopamine is released within the SNr by dendrites of pars compacta neurons (Cheramy et al., 1979; 1981). Nigral dopamine is believed to enhance GABA release from striatonigral efferents through presynaptic D_1 DA receptors (Robertson, 1992a), reducing GABA transmission in the ventromedial thalamus (Gauchy et al, 1987) and increasing locomotor activity (Jackson and Kelly, 1983a; 1983b). Furthermore, there is evidence that L-Dopa-induced rotational behaviour is dependent on both striatal and nigral mechanisms (Robertson and Robertson, 1989). This observation is compatible with studies of intranigral dopaminergic grafts which have been shown to provide some recovery in D_1, D_2 or D_1/D_2 DA receptor agonist-induced rotations, but not amphetamine-induced rotational behaviour (Robertson et al., 1991; Nikkhah et al., 1994a; Olsson et al., 1995; Mendez et al, 1996; Yurek et al., 1997).

Although we have shown that the intranigral graft has a role in the functional recovery of transplanted animals, the mechanism by which the intranigral graft exerts that

role is not clear. It is possible that the intranigral graft restores DAergic innervation to nigra-innervated structures that are not reinnervated by the intrastriatal graft, such as the STN. This notion is supported by a recent study in which *c-fos* immunoreactivity was quantified in several basal ganglia structures in rats receiving intrastriatal DAergic grafts. In those animals only the STN and GP remain overactive after transplantation and the authors concluded that the striatal graft had failed to influence those structures (Nakao et al., 1998). The STN is particularly important in basal ganglia function and has been observed to be overactive in animal models of PD (Bergman et al., 1994; Hassani et al., 1996; Nakao et al., 1998). Inactivation of the STN has been shown to reduce behavioural deficits in human Parkinson patients (Krack et al., 1997a; 1997b; 1998a; 1998b; Kumar et al., 1998a; 1998b; 1999; Limousin et al., 1998; Brown et al., 1999; Moro et al., 1999; Yokoyama et al., 1999). It has previously been demonstrated that the STN receives a nigral-derived dopaminergic innervation (Lavoie et al., 1989; Hassani et al., 1997; Cossette et al., 1999; Hedreen, 1999). DA is believed to exert an inhibitory control on STN neurons through D_1 and D_2 receptors (Campbell et al., 1985; Hassani and Feger, 1999). Thus, DAergic reinnervation of the STN may be important for reducing the activity of this structure and providing enhanced functional recovery. We are currently investigating the extent of DAergic reinnervation to the STN in double grafted animals and the possible effect of 6-OHDA lesions of the intranigral graft on STN activity.

Reinnervation of both the striatum and the SN by FVM double grafts may allow restoration of DAergic circuitry in the basal ganglia. It is well known that an intrastriatal graft alone can restore rotational symmetry in 6-OHDA-lesioned rats which is also seen in this study, in the single intrastriatal-grafted groups. However, an issue to be resolved is

the observation that the intrastriatal graft alone was not sufficient to maintain rotational symmetry in double grafted animals in which the nigral graft was subsequently damaged. It is possible that rotational symmetry in double grafted animals may be a result of the reestablishment of DAergic regulation of the nigrostriatal circuitry by both grafts (Mendez et al., 1996; Mendez and Hong 1997). The removal of one graft, in this case the intranigral graft may produce a break in the circuitry resulting in loss of the beneficial functional effect. Restoration of basal ganglia circuitry may be necessary for more complex behavioral recovery such as forelimb akinesia, sensorimotor orientation and disengage behaviour in animal models of PD, which may be more relevant to the human condition. Although restoration of DAergic regulation of the nigrostriatal circuitry may be beneficial in the functional recovery of more complex sensorimotor function, restoration of GABAergic reinnervation may also be important (Winkler et al., 1999).

In our experiment it is possible that some degree of GABAergic reinnervation may have occurred in the SN by the intranigral graft as transplanted nigral tissue likely contains GABAergic cells from the SNr (Hattori et al., 1973; Ribak et al., 1976; DiChiara et al., 1979; Ficalora and Mize, 1989). In a recent study, Winkler and colleagues (1999) observed that rats with intrastriatal dopaminergic and intranigral GABAergic grafts had a significant attenuation of deficits in the forelimb akinesia test which was more pronounced than in animals with intrastriatal DAergic grafts alone.

In summary, the results of this study suggest that the intranigral graft has an important role in the behavioural recovery of double grafted animals. Restoration of DAergic and possible GABAergic reinnervation to the striatum, SN and other nigra-

innervated structures such as the STN may be crucial for optimizing functional efficacy in neural transplantation for PD.

CHAPTER 4:

GENERAL DISCUSSION

Summary of the work

The main findings of this work are; 1) hNT neuronal grafts survive when transplanted into the rodent model of PD. Furthermore, hNT neurons express TH and hold promise as a possible alternative cell source for transplantation; and 2) simultaneous intrastriatal and intranigral grafts appear to be a superior strategy for transplantation based on previous work by our laboratory (Mendez et al., 1996; Mendez and Hong, 1997) and the substantia nigra is an appropriate and important target for transplantation in the rat model of PD.

Induction of TH expression in non-catecholaminergic cells

In chapter 2, TH immunohistochemical analysis of hNT neuronal grafts revealed a small number of THir cells. The ability of hNT neurons to provide functional recovery in the rat model of PD was described. A trend towards a reduction in amphetamine-induced rotational behaviour was observed, but never reached significance. Although a measurement of DA release was not performed or double-labeling for the presence of dopa decarboxylase (DDC) (enzyme responsible for the conversion of L-Dopa to DA), it was hypothesized that the poor functional recovery of the animals may relate to the relatively few number of cells expressing TH. Research investigating the induction of TH in non-catecholaminergic cells has resulted in the identification of various molecules and approaches to induce TH expression.

The optimal tissue source for transplantation may be one that is capable of producing and releasing DA and is neuronal in origin, capable of reinnervating DA-depleted areas of the brain. One way to induce TH expression by non-catecholaminergic

cells is through the insertion of a foreign TH transgene. Transplantation of cells with a foreign TH transgene in the rat model of PD have been observed to produce only short-term functional recovery (Wolff et al., 1989; Horellou et al., 1990a; 1990b; Fisher et al., 1991; Ishida et al., 1996b; Lundberg et al., 1996; Tornatore et al., 1996; Raymon et al., 1997; Leff et al., 1998; Fitoussi et al., 1998; Segovia et al., 1998), possibly due to a down-regulation of foreign transgene expression (Leff et al., 1998; Ljungberg et al., 1999; Trejo et al., 1999). The transgene may need to be inserted within the host DNA, in a way enabling transcription of the transgene to be under the control of the promoter of a constitutively expressed gene ensuring long-term transcription of the transgene (Trejo et al., 1999). Insertion of foreign transgenes in the hNT genome has been reported (Trojanowski et al., 1997; Kofler et al., 1998). Thus, this may be one way of producing a stable TH-expressing hNT neuronal population. If hNT neurons exhibit long-term expression of the TH transgene, further studies will be required to assess whether hNT neurons are capable of synthesizing DA. An earlier study by Imaoka and colleagues (1998), reported greater functional recovery in hemiparkinsonian rats following intrastriatal virus-mediated co-transfer of both TH and DDC transgenes than when the TH gene was transferred alone. Thus, co-transfection of hNT neurons with both TH and DDC transgenes may be required for greater recovery.

A great deal of research has been generated on the factors responsible for catecholaminergic neuronal differentiation. Factors such as sonic hedgehog (SHH) protein, aFGF and basic fibroblast growth factor (bFGF), BDNF and LiCl have all been reported to either enhance or induce TH expression in catecholaminergic and/or non-catecholaminergic neurons, respectively. The *SHH* gene encodes a protein that is

prevalent within the ventral midline of the developing CNS (Johnson et al., 1994; Ekker et al., 1995). That protein has been observed to be sufficient in inducing DAergic and other neuronal phenotypes in chick VM cultures (Wang et al., 1995). Wang and colleagues (1995) hypothesized that SHH protein is a general ventralizing signal and the phenotype induced by SHH may be determined by the receiving cells. Furthermore, the transcription factor, Nurr1 has been reported to induce TH transcription in hippocampal neural progenitors independent of the presence of SHH protein by binding a response element within the region of the *TH* gene (Sakurada et al., 1999). Furthermore, coculturing multipotent neural stem cells overexpressing Nurr1 led to greater than 80% of cells expressing a phenotype indistinguishable from midbrain DAergic neurons (Wagner et al., 1999). Saucedo-Cardenas and colleagues (1998) have reported that although SHH drives neural progenitors towards a midbrain DAergic phenotype, Nurr1 is essential for inducing a full midbrain DAergic phenotype from mesencephalic precursors. Although the complete pathway in which induction of a nigral DAergic neuronal phenotype has yet to be clearly described those results are promising. As the experiments in chapter 2 were conducted with terminally differentiated neurons, the ability of up-regulation of Nurr1 or SHH protein expression to induce a DAergic phenotype in differentiated cell lines still needs to be addressed.

The highest expression of bFGF in rat VM is observed from E16 to postnatal day 90 (P90) and of aFGF from P20 to P90 suggesting that aFGF and bFGF may have functions in midbrain DAergic neurons at different developmental stages (Bean et al., 1992). Thus, the FGF family of molecules may also be important for inducing or maintaining a DAergic phenotype in VM neurons. In 1989, Iacovitti and colleagues

(1989) reported a 20-fold increase in catecholaminergic phenotype expression (THir) in cultured rat cortical cells in the presence of factors extracted from muscle, referred to as muscle-derived factor (MDF). Furthermore, treatment of cerebellar and striatal neurons and cells from the collicular plate of the adult rat brain with MDF induced similar increases in TH expression (Iacovitti, 1991). aFGF was later found to be an important component of MDF (Du et al., 1994) and further studies revealed that DA, protein kinase A (PKA) and PKC pathway activators work synergistically to upregulate TH expression and activity in DAergic and non-DAergic neurons (Stull and Iacovitti, 1996; Du and Iacovitti, 1997a; 1997b). That mixture of factors is hypothesized to induce the phosphorylation of mitogen activated protein kinase through FGF receptors and increased transcription factor binding of the AP-1 regulatory element of the *TH* gene and a concomitant decrease in levels of repressor proteins, effectively enhancing TH expression (Guo et al., 1998). Regardless of the mechanism, exposure of the DA-denervated striatum of MPTP-treated mice to those factors for 14 days significantly enhances TH activity (Jin and Iacovitti, 1996). Furthermore, in the 6-OHDA rat model of PD, intrastriatal infusion of those factors significantly reduces amphetamine-induced rotational behaviour, for up to 8 weeks, the longest time period tested following infusion (Jin and Iacovitti, 1995). The results of those studies are very promising, outlining a technique to induce TH expression in non-catecholaminergic cells, however treatment of hNT neurons with the above mixture provides only a small increase in the number of THir neurons (Iacovitti and Stull, 1997).

LiCl induces TH expression in frontal cortex, hippocampus and striatum in adults rats following acute and chronic treatment, *in vivo* (Chen et al., 1998). LiCl promotes a

similar enhancement of TH expression in cutured human SH-SY5Y neuroblastoma and bovine adrenal medullary cells (Terao et al., 1992; Chen et al., 1998) but inhibits TH expression in pheochromocytoma-12 cells (Presse et al., 1997). LiCl enhances the activity of PKA (Terao et al., 1992) and regulates TH expression through the AP-1 transcription factor family (Chen et al., 1998). Furthermore, Zigova and colleagues (1999) reported a significant increase of TH expression in hNT neurons in culture following LiCl treatment. Our results suggest that LiCl pretreatment of hNT-DA neurons may enhance the number of cells expressing TH following transplantation. In our experiments all animals (n=4) with LiCl pretreated hNT-DA neuronal grafts contatined THir cells compared to 43% (n=3 of 7) of animals with untreated hNT-DA neurons. Stimulation of several signalling pathways (ie., PKA and calcium calmodulin-dependent kinase pathways) may be required to enhance TH expression in hNT neurons (Nankova et al., 1996). Further studies are required to assess whether treatment of neurons with the above factors not only enhance TH expression but may concomitantly increase dopa decarboxylase (DDC) expression and thus, DA production which may afford greater clinical benefit than simply L-Dopa-producing cells. DA-producing cells may be important as the disease progresses as a mechanism to convert L-Dopa to DA may not be available as the endogenous neurons continue to degenerate.

In summary, hNT neurons hold promise as an alternative source of cells for transplantation in PD. However, increasing their ability to express TH is critical for hNT cells to become a practical alternative to fetal VM tissue.

Double DAergic grafts in the rat Parkinson model

Our laboratory has previously reported on the increased functional effects of simultaneous intrastriatal and intranigral FVM grafts compared to intrastriatal grafts alone (Mendez et al., 1996). To determine the mechanism by which double grafts may enhance amphetamine-induced rotational recovery, Mendez and Hong (1997) performed a tracer study using fluorogold (FG) and HRP. Following intrastriatal FG injections, 11.5% of THir cells within the intranigral graft were also fluorescent. Those results suggest that enhanced rotational recovery in double grafted animals may be partially explained by increased striatal reinnervation, as the extent of striatal reinnervation correlates well with the degree of functional recovery (Rioux et al., 1991; Apostolides et al., 1998; Winkler et al., 1999). Although increased striatal reinnervation may partially explain the results, previous studies indicate that the SN may also be important for functional recovery (Robertson, 1992b). Dendritic release of DA within the SNr enhances the release of GABA by the descending striatonigral pathway (Robertson, 1992a). Furthermore, infusion of DA or DA agonists within the SN reduces GABA release in the thalamus (Gauchy et al., 1987) and results in an overall increase in locomotor activity in rats (Jackson and Kelly, 1983a; 1983b). Therefore, DAergic reinnervation of the SNr may be necessary for regulating the inhibitory drive of SNr projection neurons on target nuclei. The regulation of SNr neuronal activity may thus be important for enhancing functional recovery and re-establishing normal basal ganglia acitvity in PD patients.

Recently, Winkler and colleagues (1999) reported on a significant amelioration of forelimb akinesia in the rat model of PD with simultaneous intrastriatal DAergic and

intranigral GABAergic grafts. However, this recovery remained incomplete. Although the double grafting strategy improved forelimb function, intrastriatal DAergic grafts were more effective in reducing amphetamine-induced rotational behaviour and equally effective to the double grafts in reducing apomorphine-induced rotational behaviour. Furthermore, in that study as well as other's, multiple intrastriatal deposits of nigral suspensions were made (18 in total) greatly enhancing the extent of striatal reinnervation (Nikkhah et al., 1993; Olsson, et al., 1995; Winkler et al., 1999). However, Winkler and colleagues (1999) reported that the density of the THir fibers in the striatum were similar in the single and double grafted groups and far greater than animals with partial lesions of the nigrostriatal pathway, however the functional effects were similar to that of partially lesioned animals. Those results suggest that complete reinnervation of the striatum may not be essential for the restoration of complex sensorimotor behaviours. Reinnervation of other DA-depleted nuclei such as the SN may be necessary for complex sensorimotor behavioural recovery.

The observations that the intranigral DAergic graft extends fibers to the ipsilateral striatum in double grafted rats (Mendez and Hong, 1997) and simultaneous intrastriatal DAergic and intranigral GABAergic grafts ameliorate forelimb akinesia (Winkler et al., 1999) made it imperative that we investigate whether the intranigral DAergic graft was truly necessary for functional recovery. In chapter 3, the role of the intranigral graft in double grafted animals is discussed. Ten weeks following transplantation, animals received intranigral vehicle or 6-OHDA injections. In double grafted animals with subsequent intranigral 6-OHDA injections, a reversal of amphetamine-induced rotational recovery was observed. That reversal of recovery was not exhibited by double grafted

rats with subsequent vehicle injections or intrastriatally grafted animals with intranigral 6-OHDA injections. Those observations rule out the possibilty that neither the trauma to the intranigral graft or SN nor the destruction of spared DAergic neurons of the first lesion led to the increase in amphetamine-induced rotational behaviour. Those results provide clear evidence that the intranigral DAergic graft is essential for functional recovery in double grafted hemiparkinsonian rats.

Although an analysis of the effects of double grafts on complex sensorimotor behavioural recovery was not performed. Preliminary results from our laboratory indicate that double DAergic grafts may promote a quicker recovery in stepping test performance, as early as 2 weeks following transplantation (Baker et al., *in preparation*). Thus, a more complete amelioration of behavioural deficits in the hemiparkinsonian rat may depend on restoring the DAergic innervation of other DA-depleted brain regions.

DA-denervated regions of the mammalian brain: Possible targets for neural transplantation?

The SN contains approximately 80% of DAergic neurons in the central nervous system. It is well known that the main target area of those DAergic neurons is the striatum (Andén et al., 1964; 1965; 1966). But further evidence has been generated suggesting that the nigral DAergic neurons also innervate other areas of the brain. Early studies revealed that THir fibers originating from the SNc / ventral tegmental area extended into the nucleus accumbens, olfactory bulb, anterior olfactory nucleus, olfactory tubercle, interstitial nucleus of the stria terminalis, lateral septal nucleus, central amygdaloid nucleus, cingulate cortex, entorhinal cortex, inferior colliculus and

hippocampus (Emson and Koob, 1978; for a review see, Moore and Bloom, 1978; Olazabal and Moore, 1989; Cheung et al., 1998; Williams and Golman-Rakic, 1998) (Figure 4.1). 6-OHDA lesions of the catecholaminergic terminals in the nucleus accumbens, central amygdaloid nucleus, olfactory bulb, hippocampus and frontal cortex have been reported to result in deficts in certain learning and memory paradigms, body weight regulation, rewarding behaviours, motivation and taste aversion based on olfactory cues (Lenard and Hahn, 1982; Fernandez-Ruíz et al., 1993; Rassnick et al., 1993; Gasbarri et al., 1996; Morrow et al., 1999). However, destruction of noradrenergic terminals in those structures can not be ruled out as a possible contributing factor to the appearance of those deficits (Lenard and Hahn, 1982). A loss of DAergic innervation of the frontal cortex has been hypothesized to possibly accentuate the depressive mood exhibited by PD patients (Fibiger, 1984).

Furthermore, 6-OHDA injections into the nucleus accumbens and central amygdaloid nucleus have been reported to result in DA agonist-induced locomotor deficits (Deminiere et al., 1988; Simon et al., 1988; Herman et al., 1988). Cotransplantation of FVM and fetal locus coeruleus, as a source of noradrenergic neurons, within the nucleus accumbens and/or frontal cortex reduced amphetamine-induced but not apomorphine-induced rotational behaviour, whereas grafts of FVM tissue alone did (Cenci et al., 1994). However, skilled forelimb use remained unaffected (Cenci et al., 1994). Abrous and colleagues (1993) reported no significant reduction in amphetamine-induced and a small reduction in apomoporphine-induced rotational behaviour by intra-accumbens nigral grafts. However, double DAergic grafts (intrastriatal and intra-accumbens) provided significant rotational recovery following

challenge with either DA agonist, however, skilled forelimb deficits again remained unaffected (Abrous et al., 1993). Other studies have reported a significant reduction in amphetamine-induced rotational recovery by intra-accumbens FVM grafts alone (Brundin et al., 1987; Abrous et al., 1990; 1993; Ishida et al., 1991).

Intrastriatal and/or intra-accumbens DAergic grafts in 6-OHDA-lesioned rats fail to completely alleviate complex sensorimotor behavioural deficits, such as skilled forelimb use, suggesting incomplete normalization of basal ganglia anatomy and physiology. Several studies have demonstrated the existence of DAergic innervation of the STN by SNc fibers (Lavoie et al., 1989; Hassani et al., 1997; Cossette et al., 1999; Hedreen, 1999). There is also evidence that intrastriatal grafts fail to normalize STN activity as indicated c-fos expression following apomorphine challenge (Nakao et al., 1998). In 6-OHDA-lesioned rats, MPTP-treated monkeys, and PD patients subthalamic inactivation promotes functional recovery (Anderson et al., 1992; Krack et al., 1997a; 1997b; 1998a; 1998b; Kumar et al., 1998a; 1998b; 1999; Limousin et al., 1998; Phillips et al., 1998; Brown et al., 1999; Moro et al., 1999; Yokoyama et al., 1999). Furthermore, in normal rats intrasubthalamic nucleus microinjections of D_1 or D_2 agonists reduce the discharge rate of STN neuronal activity, whereas in 6-OHDA-lesioned animals, D_1 agonists but not D_2 agonists reduce neuronal discharge rates in the STN (Hassani and Feger, 1999). Blockade of STN D_1 but not D_2 DA receptors in normal rats induces akinesia (Hauber, 1998). Although it is not completely clear whether intrasubthalamic DA has a net inhibitory or excitatory effect on subthalamic neurons, it is possible that the STN may also be an important target site for DAergic grafts in PD. Currently, the ability of double DAergic grafts (intrastriatal and intranigral) to normalize c-fos expression in

the STN is being investigated in our laboratory. The results of that study should elucidate whether the STN may be a possible target for transplantation in PD models.

It is possible that several DA-denervated targets may need to be transplanted in the basal ganglia for neural transplantation strategies to produce sustained beneficial effects in Parkinsonian patients. It is clear that the current strategy of reinnervating the striatum by ectopically placed DAergic grafts has not reached the clinical efficacy for neural transplantation to be used as a routine therapeutic procedure for PD. Our work promotes the idea of a multi-target transplantation strategy, which may have important clinical implications in the future.

Figure 4.1 – Schematic diagram of the rat brain demonstrating the target nuclei of the DAergic projecton neurons of the substantia nigra (SN) / ventral tegmental area (VTA). Am, amygdala; CC, cingulate cortex; EC, entorhinal cortex; EPN, entopeduncular nucleus; FC, frontal cortex; GP, globus pallidus; HPC, hippocampus; IC, inferior colliculus; NAc, nucleus accumbens; OB, olfactory bulb; SA, septal area; ST, stria terminalis; STN, subthalamic nucleus; STR, striatum.

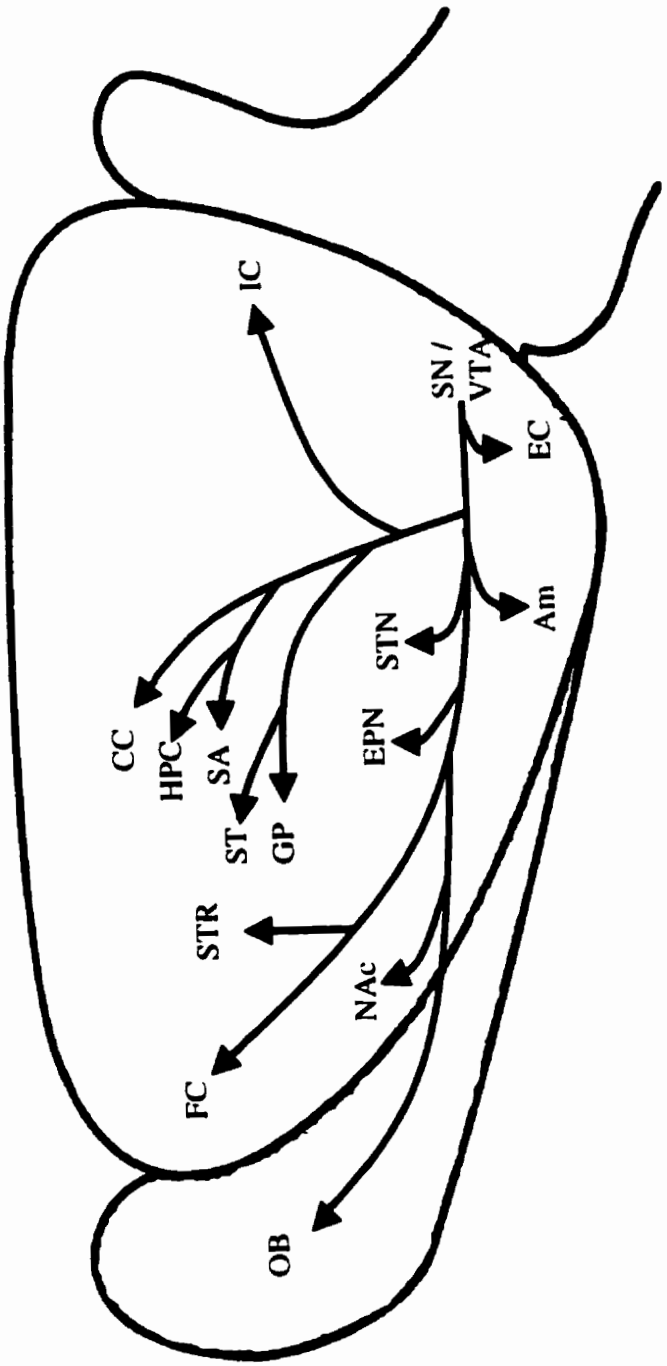

Figure 4.1

Future Perspectives

The possible future perspective of the work described in this thesis are as follows:

1. hNT neurons were evaluated as a possible abundant alternative cell source for transplantation. However, before hNT neurons can be considered as a possible alternative to fetal tissue for transplantation, a technique must be developed to induce the long-term expression of TH in those cells. That technique may involve the transfection of hNT neurons with foreign TH transgenes or treating the neurons with several different factors inducing differentiation of the hNT neurons into DAergic neurons. If a cell line can be established exhibiting high levels of TH expression, the next step would be to establish whether they synthesize and release DA and promote functional recovery in the rat model of PD.

2. The optimal placement site for DAergic grafts was also addressed in this paper by investigating whether the intranigral DAergic graft was truly essential for functional recovery in double grafted rats. The observation that 6-OHDA lesions of the intranigral DAergic graft in double grafted animals reverse the functional recovery obtained following transplantation is interesting for several reasons. First, those results demonstrate the importance of the intranigral graft in maintaining functional recovery in double grafted rats. Intrastriatal DAergic grafts alone are sufficient to provide amphetamine-induced rotational recovery in 6-OHDA-lesioned rats. However, the observation that the second 6-OHDA lesion reverses the recovery in double grafted animals, suggests that removal of the intranigral graft possibly results in changes within

the basal ganglia nuclei which are not compensated for by the presence of the intrastriatal graft. Presently, our laboratory is utilizing c-fos immunohistochemistry to elucidate which structures within the basal ganglia may exhibit abnormal neuronal activity following 6-OHDA lesions of the intranigral graft.

Our laboratory is presently investigating whether other DA denervated areas of the brain, such as the STN may also be important target sites for neural transplantation to alleviate the complex sensorimotor deficits observed in animal models of PD. It is possible that a multi-target grafting strategy may be a superior strategy in neural transplantation for PD. The results of those experiments may prove important for the future of neural transplantation in PD.

BIBLIOGRAPHY

Abbas N, Lucking CB, Ricard S, Durr A, Bonifati V, De Michele G, Bouley S, Vaughan JR, Gasser T, Marconi R, Broussolle E, Brefel-Courbon C, Harhangi BS, Oostra BA, Fabrizio E, Bohme GA, Pradier L, Wood NW, Filla A, Meco G, Denefle P, Agid Y, Brice A (1999) A wide variety of mutations in the parkin gene are responsible for autosomal recessive parkinsonism in Europe. French Parkinson's Disease Genetics Study Group and the European Consortium on Genetic Susceptibility in Parkinson's Disease. *Hum Mol Genet* 8: 567-574

Abercrombie M (1946) Estimation of nuclear population from microtome sections. *Anat Rec* 94: 239-247

Abraham I, Sampson KE, Powers EA, Mayo JK, Ruff VA, Leach KL (1991) Increased PKA and PKC activities accompany neuronal differentiation of NT2/D1 cells. *J Neurosci Res* 28: 29-39

Abrous DN, Le Moal M, Herman JP (1994) The increase in striatal neuropeptide Y immunoreactivity induced by neonatal dopamine-depleting lesions in rats is reversed by intrastriatal dopamine-rich transplants. *Brain Res* 656: 169-173

Abrous DN, Shaltot AR, Torres EM, Dunnett SB (1993) Dopamine-rich grafts in the neostriatum and/or nucleus accumbens: Effects on drug-induced behaviours and skilled paw-reaching. *Neuroscience* 53: 187-197

Adams FS, La Rosa FG, Kumar S, Edwards-Prasad J, Kentroti S, Vernadakis A, Freed CR, Prasad KN (1996) Characterization and transplantation of two neuronal cell lines with dopaminergic properties. *Neurochem Res* 21: 619-627

Alarcon F, Cevallos N, Lees AJ (1998) Does combined levodopa and bromocriptine therapy in Parkinson's disease prevent late motor complications? *Eur J Neurol* 5: 255-263

Albin RL, Young AB, Penney JB (1989) The functional anatomy of basal ganglia disorders. *Trends Neurosci* 12: 366-375

Allain H, Cougnard J, Neukirch H-C , FSMT members (1991) Selegiline in *de novo* Parkinsonian patients: the French selegiline multicenter trial (FSMT). *Acta Neurol Scand Suppl* 84: 73-78

Allen GS, Burns RS, Tulipan NB, Parker RA (1989) Adrenal medullary transplantation to the caudate nucleus in Parkinson's disease. *Arch Neurol* 46: 487-491

Andén N-E, Carlsson A, Dahlström A, Fuxe K, Hillarp N-A, Larsson K (1964) Demonstration and mapping out of nigro-neostriatal dopamine neurons. *Life Sci* 3: 523-530

Andén N-E, Dahlström A, Fuxe K, Larsson K (1965) Further evidence for the presence of nigro-neostriatal neurons in the rat. *Am J Anat* 116: 329-334

Andén N-E, Dahlström A, Fuxe K, Larsson K, Olson L, Ungerstedt U (1966) Ascending monoamine neurons to the telencephalon and diencephalon. *Acta Physiol Scand* 67: 313-326

Anderson JJ, Chase TN, Engber TM (1992) Differential effect of subthalamic nucleus ablation on dopamine D1 and D2 agonist-induced rotation in 6-hydroxydopamine-lesioned rats. *Brain Res* 588: 307-310

Andrews PW (1984) Retinoic acid induces neuronal differentiation of a cloned human embryonal carcinoma cell line *in vitro*. *Dev Biol* 103: 285-293

Andrews PW (1987) Human teratocarcinoma stem cells: Glycolipid antigen expression and modulation during differentiation. *J Cell Biochem* 35: 321-332

Annett LE, Torres EM, Clarke DJ, Ishida Y, Barker RA, Ridley RM, Baker HF, Dunnett SB (1997) Survival of nigral grafts within the striatum of marmosets with 6-OHDA lesions depends critically on donor embryo age. *Cell Transplant* 6: 557-569

Ansari KS, Yu PH, Kruck TPA, Tatton WG (1993) Rescue of axotomized immature rat facial motoneurons by R-(-)-deprenyl: Stereospecificity and independeance from monoamine oxidase inhibition. *J Neurosci* 13: 4042-4053

Apostolides C, Sanford E, Hong M, Mendez I (1998) Glial cell line-derived neurotrophic factor improves intrastriatal graft survival of stored dopaminergic cells. *Neuroscience* 83: 363-372

Apuzzo MLJ, Neal JH, Waters CH, Appley AJ, Boyd SD, Couldwell WT, Wheelock VH, Weiner LP (1990) Utilization of unilateral and bilateral stereotactically placed adrenomedullary-striatal autografts in parkinsonian humans: Rationale, techniques, and observations. *Neurosurgery* 26: 746-757

Backlund EO, Granberg PO, Hamberger B, Knutsson E, Martensson A, Sedvall G, Seiger Å, Olson L (1985) Transplantation of adrenal medullary tissue to striatum in parkinsonism. First clinical trials. *J Neurosurg* 62: 169-173

Bakay RAE (1989) Preliminary report on adrenal medullary grafting from the American Association of Neurological Surgeons GRAFT project. *Restorative Neurol Neurosci* 1: 158

Bal A, Savasta M, Chritin M, Mennicken F, Abrous DN, Le Moal M, Feuerstein C, Herman JP (1993) Transplantation of fetal nigral cells reverses the increase of preproenkephalin mRNA levels in the rat striatum caused by 6-OHDA lesion of the dopaminergic nigrostriatal pathway: a quantitative *in situ* hybridization study. *Brain Res Mol Brain Res* 18: 221-227

Baron MS, Vitek JL, Bakey RAE, Green J, Kaneoke Y, Hashimoto T, Turner RS, Woodard JL, Cole SA, McDonald WM, DeLong MR (1996) Treatment of advanced Parkinson's disease by posterior GPi pallidotomy: 1 year results of a pilot study. *Ann Neurol* 40: 355-366

Barone P, Bravi D, Bermejo-Pareja F, Marconi R, Kulisevsky J, Malagu S, Weiser R, Rost N (1999) Pergolide monotherapy in the treatment of early PD: A randomized, controlled study. Pergolide Monotherapy Study Group. *Neurology* 53: 573-579

Bayulkem K, Erisir K, Tuncel A, Bayulkem B (1996) A study on the effect and tolerance of lisuride on Parkinson's disease. *Adv Neurol* 69: 519-30

Bean AJ, Oellig C, Pettersson RF, Hokfelt T (1992) Differential expression of acidic and basic FGF in the rat substantia nigra during development. *Neuroreport* 3: 993-996

Benabid AL, Pollak P, Gao D, Hoffmann D, Limousin P, Gay E, Payen I, Benazzouz A (1996) Chronic electric stimulation of the ventralis intermedius nucleus of the thalamus as a treatment of movement disorders. *J Neurosurg* 84: 203-214

Benabid AL, Pollak P, Gervason C, Hoffmann D, Gao DM, Hommel M, Perret JE, de Rougemont J (1991) Long-term suppression of tremor by chronic stimulation of the ventral intermediate thalamic nucleus. *Lancet* 337: 403-406

Bennett WM (1998) The nephrotoxicity of new and old drugs. *Ren Fail* 20: 687-690

Bergman H, Wichmann T, Karmon B, Delong MR (1994) The primate subthalamic nucleus. II. Neuronal activity in the MPTP model of Parkinsonism. *J Neurophysiol* 72: 507-520

Bickford ME, Hall WC (1992) The nigral projection to predorsal bundle cells in the superior colliculus of the rat. *J Comp Neurol* 319: 11-33

Bilang-Bleuel A, Revah F, Colin P, Locquet I, Robert JJ, Mallet J, Horellou P (1997) Intrastriatal injection of an adenoviral vector expressing glial cell line-derived neurotrophic factor prevents dopaminergic neuron degeneration and behavioral impairment in a rat model of Parkinson disease. *Proc Natl Acad Sci USA* 94: 8818-8823

Bing GY, Notter MF, Hansen JT, Gash DM (1988) Comparison of adrenal medullary, carotid body and PC12 cell grafts in 6-OHDA lesioned rats. *Brain Res Bull* 20: 399-406

Björklund A, Dunnett SB, Stenevi U, Lewis ME, Iversen SD (1980) Reinnervation of the denervated striatum by substantia nigra transplants: Functional consequences as revealed by pharmacological and sensorimotor testing. *Brain Res* 199: 307-333

Björklund A, Kramer LF, Stenevi U (1979) Cholinergic reinnervation of rat hippocampus by septal implants is stimulated by perforant path lesion. *Brain Res* 173: 57-64

Björklund A, Stenevi U (1977) Reformation of the severed septohippocampal cholinergic pathway in the adult rat by transplanted septal neurons. *Cell Tissue Res* 185: 289-302

Björklund A, Stenevi U (1979) Reconstruction of the nigrostriatal dopamine pathway by intracerebral nigral transplants. *Brain Res* 177: 555-560

Björklund A, Stenevi U, Schmidt RH, Dunnett SB, Gage FH (1983) Intracerebral grafting of neuronal cell suspensions. II. Survival and growth of nigral cell suspensions implanted in different brain sites. *Acta Physiol Scand Suppl* 522: 9-18

Björklund A, Stenevi U, Svendgaard NA (1976) Growth of transplanted monoaminergic neurones into the adult hippocampus along the perforant path. *Nature* 262: 787-790

Bluml S, Kopyov O, Jacques S, Ross BD (1999) Activation of neural transplants in humans. *Exp Neurol* 158: 121-125

Boecker H, Wills, AJ, Ceballos-Bauman A (1997) Stereotactic thalamotomy in tremor-dominant Parkinson's disease: An $H_2{}^{15}O$ PET motor activation study. *Ann Neurol* 41: 108-111

Boraud T, Bezard E, Bioulac B, Gross C (1996) High frequency stimulation of the internal Globus Pallidus (GP) simultaneously improves Parkinsonian symptoms and reduces the firing frequency of GPi neurons in the MPTP-treated monkey. *Neurosci Lett* 215: 17-20

Borlongan CV, Saporta S, Poulos SG, Othberg A, Sanberg PR (1998a) Viability and survival of hNT neurons determine degree of functional recovery in grafted ischemic rats. *Neuroreport* 9: 2837-2842

Borlongan CV, Tajima Y, Trojanowski JQ, Lee VM-Y, Sanberg PR (1998b) Transplantation of cryopreserved human embryonal carcinoma-derived neurons (NT2N cells) promotes functional recovery in ischemic rats. *Exp Neurol* 149: 310-321

Brannan T, Yahr MD (1995) Comparative study of selegiline plus L-Dopa-carbidopa versus L-Dopa-carbidopa alone in the treatment of Parkinson's disease. *Ann Neurol* 37: 95-98

Brooks DJ, Abbott RJ, Lees AJ, Martignoni E, Philcox DV, Rascol O, Roos RA, Sagar HJ (1998) A placebo-controlled evaluation of ropinirole, a novel D2 agonist, as sole dopaminergic therapy in Parkinson's disease. *Clin Neuropharmacol* 21: 101-107

Brown RG, Dowsey PL, Brown P, Jahanshahi M, Pollak P, Benabid AL, Rodriguez-Oroz MC, Obeso J, Rothwell JC (1999) Impact of deep brain stimulation on upper limb akinesia in Parkinson's disease. *Ann Neurol* 45: 473-488

Brown VJ, Dunnett SB (1989) Comparison of adrenal and foetal nigral grafts on drug induced rotation in rats with 6-OHDA lesions. *Exp Brain Res* 78: 214-218

Brundin P, Nilsson OG, Strecker RE, Lindvall O, Åstedt B, Björklund A (1986) Behavioural effects of human fetal dopamine neurons grafted in a rat model of Parkinson's disease. *Exp Brain Res* 65: 235-240

Brundin P, Strecker RE, Londos E, Björklund A (1987) Dopamine neurons grafted unilaterally to the nucleus accumbens affect drug-induced circling and locomotion. *Exp Brain Res* 69: 183-194

Brundin P, Strecker RE, Widner H, Clarke DJ, Nilsson OG, Åstedt B, Lindvall O, Björklund (1988) Human fetal dopamine neurons grafted in a rat model of Parkinson's disease: Immunological aspects, spontaneous and drug-induced behaviour, and dopamine release. *Exp Brain Res* 70: 192-208

Butler D (1998) Briefing xenotransplantation. Last chance to stop and think on risks of xenotransplants. *Nature* 391: 320-324

Cadet JL, Zhu SM, Angulo JA (1991) Intrastriatal implants of fetal mesencephalic cells attenuate the increases in striatal proenkephalin mRNA observed after unilateral 6-hydroxydopamine-induced lesions of the striatum. *Brain Res Bull* 27: 707-711

Campbell GA, Eckardt MJ, Weight FF (1985) Dopaminergic mechansims in subthalamic nucleus of the rat: analysis using horseradish peroxidase and microiontophoresis. *Brain Res* 333: 261-270

Carter CJ (1982) Topographical distribution of possible glutamatergic pathways from the frontal cortex to the striatum and substantia nigra in rats. *Neuropharmacology* 21: 379-383

Cenci MA, Campbell K, Björklund (1993) Neuropeptide messenger RNA expression in the 6-hydroxydopamine-lesioned rat striatum reinnervated by fetal dopaminergic transplants: Differential effects of the grafts on preproenkephalin, preprotachykinin and prodynorphin messenger RNA levels. *Neuroscience* 57: 275-296

Cenci MA, Campbell K, Björklund A (1997) Glutamic acid decarboxylase gene expression in the dopamine-denervated striatum: Effects of intrastriatal fetal nigral transplants or chronic apomorphine treatment. *Brain Res Mol Brain Res* 48: 149-155

Cenci MA, Kalen P, Duan WM, Björklund A (1994) Transmitter release from transplants of fetal ventral mesencephalon or locus coeruleus in the rat frontal cortex and nucleus accumbens: Effects of pharmacological and behaviorally activating stimuli. *Brain Res* 641: 225-248

Chen G, Yuan P-X, Jiang Y-M, Huang L-D, Manji HK (1998) Lithium increases tyrosine hydroxylase levels both *in vivo* and *in vitro*. *J Neurochem* 70: 1768-1771

Cheramy A, Leviel V, Glowinski J (1981) Dendritic release of dopamine in the substantia nigra. *Nature* 289: 537-542

Cheramy A, Nieoullon A, Glowinski J (1979) *In vivo* evidence for a dendritic release of dopamine in cat substantia nigra. *Appl Neurophysiol* 42: 57-59

Cheung S, Ballew JR, Moore KE, Lookingland KJ (1998) Contribution of dopamine neurons in the medial zona incerta to the innervation of the central nucleus of the amygdala, horizontal diagonal band of Broca and hypothalamic paraventricular nucleus. *Brain Res* 808: 174-181

Chiueh CC, Huang S-J, Murphy DL (1994) Suppression of hydroxyl radical formation by MAO inhibitors: A novel possible neuroprotective mechanism in dopaminergic neurotoxicity. *J Neural Transm* 41 (Suppl): 189-196

Clarke DJ, Brundin P, Strecker RE, Nilsson OG, Björklund A (1988) Human fetal dopamine neurons grafted in a rat model of Parkinson's disease: Ultrastructural evidence for synapse formation using tyrosine hydroxylase immunocytochemistry. *Exp Brain Res* 73: 115-126

Clarke PBS, Hommer DW, Pert A, Skirroll LR (1987) Innervation of substantia nigra neurons by cholinergic afferents from pedunculopontine nucleus in the rat: Neuroanatomical and electrophysiological evidence. *Neuroscience* 23: 1011-1019

Clayton DF, George JM (1999) Synucleins in synaptic plasticity and neurodegenerative disorders. *J Neurosci Res* 58: 120-129

Cohen G, Spina MB (1989) Deprenyl suppresses the oxidant stress associated with increased dopamine turnover. *Ann Neurol* 26: 689-690

Cook DG, Forman MS, Sung JC, Leight S, Kolson DL, Iwatsubo T, Lee VM-Y, Doms RW (1997) Alzheimer's A β(1-42) is generated in the endoplasmic reticulum/intermediate compartment of NT2N cells. *Nat Med* 3: 1021-1023

Cook, DG, Turner RS, Kolson DL, Lee VM-Y, Doms RW (1996)Vaccinia virus serves as an efficient vector for expressing heterologous proteins in human NTera 2 neurons. *J Comp Neurol* 371: 481-492

Cossette M, Levesque M, Parent A (1999) Extrastriatal dopaminergic innervation of human basal ganglia. *Neurosci Res* 34: 51-54

Das GD (1974) Transplantation of embryonic neural tissue in the mammalian brain. Growth and differentiation of neuroblasts from various regions of the brain in the cerebellum of neonate rats. *J Life Sci* 4: 93-124

Davis KD, Taub E, Houle S, Lang AE, Dostrovsky JO, Tasker RR, Lozano AM (1997) Globus pallidus stimulation activates the cortical motor system during alleviation of Parkinsonian symptoms. *Nat Med* 3: 671-674

Deacon T, Dinsmore J, Costantini LC, Ratliff J, Isacson O (1998) Blastula-stage stem cells can differentiate into dopaminergic and serotonergic neurons after transplantation. *Exp Neurol* 149: 28-41

Deacon TW, Schumacher TJ, Dinsmore J, Thomas C, Palmer P, Kott S, Edge A, Penney D, Kassissieh S, Dempsey P, Isacson O (1997) Histological evidence of fetal pig neural cell survival after transplantation into a patient with Parkinson's disease. *Nat Med* 3: 350-353

Defebvre L, Blatt J-L, Blond S, Bourriez J-L, Guieu J-D, Destee A (1996) Effect of thalamic stimulation on gait in Parkinson's disease. *Arch Neurol* 53: 898-903

Defer G-L, Geny C, Ricolfi F, Fenelon G, Monfort J-C, remy P, Villafane G, Jeny R, Samson Y, Keravel Y, Gaston A, Degos J-D, Peschanski M, Cesaro P, N'Guyen J-P (1996) Long-term outcome of unilaterally transplanted Parkinsonian patients. I. Clinical approach. *Brain* 119: 41-50

DeLong MR, Alexander GE, Mitchell SJ, Richardson RT (1986) The contribution of basal ganglia to limb control. *Prog Brain Res* 64: 161-174

Deminiere JM, Taghzouti K, Tassin JP, Le Moal M, Simon H (1988) Increased sensitivity to amphetamine and facilitation of amphetamine self-administration after 6-hydroxydopamine lesions of the amygdala. *Psychopharmacology* 94: 232-236

DiChiara G, Porceddu ML, Morelli M, Mulas M, Gessa GL (1979) Evidence for a GABAergic projection from the substantia nigra to the ventromedial thalamus and to the superior colliculus of the rat. *Brain Res* 176: 273-284

Dinsmore JH, Deacon TW, Isacson O Fetal neural xenografts as therapy for Parkinson's and Huntington's disease. *Biotech Int* (in press)

Dogali M, Fazzini E, Kolodny E, Eidelberg D, Sterio D, Devinsky O, Beric A (1995) Stereotactic ventral pallidotomy for Parkinson's disease. *Neurology* 45: 753-761

Doucet G, Murata Y, Brundin P, Bosler O, Mons N, Geffard M, Ouimet CC, Björklund A (1989) Host afferents into intrastriatal transplants of fetal ventral mesencephalon. *Exp Neurol* 106: 1-19

Du X, Iacovitti L (1997a) Multiple signaling pathways direct the initiation of tyrosine hydroxylase gene expression in cultured brain neurons. *Brain Res Mol Brain Res* 50: 1-8

Du X, Iacovitti L (1997b) Protein kinase C activators work in synergy with specific growth factors to initiate tyrosine hydroxylase expression in striatal neurons in culture. *J Neurochem* 68: 564-569

Du X, Stull ND, Iacovitti L (1994) Novel expression of the tyrosine hydroxylase gene requires both acidic fibroblast growth factor and an activator. *J Neurosci* 14: 7688-7694

Du X, Stull ND, Iacovitti L (1995) Brain-derived neurotrophic factor works coordinately with partner molecules to initiate tyrosine hydroxylase expression in striatal neurons. *Brain Res* 680: 229-233

Dunn EH (1917) Primary and secondary findings in a series of attempts to transplant cerebral cortex in albino rats. *J Comp Neurol* 22: 565-582

Dunnett SB, Björklund A, Schmidt RH, Stenevi U, Gage FH (1983a) Intracerebral grafting of neuronal cell suspensions. IV. Behavioural recovery in rats with unilateral 6-OHDA lesions following implantation of nigral cell suspensions in different forebrain sites. *Acta Neurol Scand Suppl* 522: 29-37

Dunnett SB, Björklund A, Schmidt RH, Stenevi U, Iversen SD (1983b) Intracerebral grafting of neuronal cell suspensions. V. Behavioural recovery in rats with bilateral 6-OHDA lesions following implantation of nigral cell suspensions. *Acta Physiol Scand Suppl* 522: 39-47

Dunnett SB, Björklund A, Stenevi U, Iversen SD (1981a) Behavioural recovery following transplantation of substantia nigra in rats subjected to 6-OHDA lesions of the nigrostriatal pathway. I. Unilateral lesions. *Brain Res* 215: 147-161

Dunnett SB, Björklund A, Stenevi U, Iversen SD (1981b) Behavioural recovery following transplantation of substantia nigra in rats subjected to 6-OHDA lesions of the nigrostriatal pathway. I. Bilateral lesions. *Brain Res* 229: 457-470

Dunnett SB, Björklund A, Stenevi U, Iversen SD (1981c) Grafts of embryonic substantia nigra reinnervating the ventrolateral striatum ameliorate sensorimotor impairments and akinesia in rats with 6-OHDA lesions of the nigrostriatal pathway. *Brain Res* 229: 209-217

Dunnett SB, Roger DC, Richards SJ (1989) Nigrostriatal reconstruction after 6-OHDA lesions in rats: Combination of dopamine-rich nigral grafts and nigrostriatal "bridge" grafts. *Exp Brain Res* 75: 523-533

Earl CD, Reum T, Xie JX, Sautter J, Kupsch A, Oertel WH, Moregenstern R (1996) Foetal nigral cell suspension grafts influence dopamine release in the non-grafted side in the 6-hydroxydopamine rat model of Parkinson's disease: I*n vivo* voltammetric data. *Exp Brain Res* 109: 179-184

Ekker SC, Ungar AR, Greenstein P, von Kessler DP, Porter JA, Moon RT, Beachy PA (1995) Patterning activities of vertebrate hedgehog proteins in the developing eye and brain. *Curr Biol* 5: 944-955

Emerich DF, Winn SR, Lindner MD (1996) Continued presence of intrastriatal but not intraventricular polymer-encapsulated PC12 cells is required for alleviation of behavioral deficits in Parkinsonian rodents. *Cell Transplant* 5: 589-596

Emson PC, Koob GF (1978) The origin and distribution of dopamine-containing afferents to the rat frontal cortex. *Brain Res* 142: 249-267

Engberg G, Elebring T, Nissbrandt H (1991) Deprenyl (Selegiline), a selective MAO-B inhibitor with active metabolites; effects on locomotor activity, dopaminergic neurotransmission and firing rate of nigral dopamine neurons. *J Pharmacol Exp Ther* 259: 841-847

Fazzini E, Dogali M, Sterio D, Eidelberg D, Beric A (1997) Stereotactic pallidotomy for Parkinson's disease: A long-term follow-up of unilateral pallidotomy. *Neurology* 48: 1273-1277

Fernandez-Ruíz J, Guzman R, Martinez MD, Miranda MI, Bermudez-Rattoni F, Drucker-Colín R (1993) Adrenal medullary grafts restore olfactory deficits and catecholamine levels of 6-OHDA amygdala lesioned animals. *J Neural Transplant Plast* 4: 289-297

Fibiger HC (1984) The neurobiological substrates of depression in Parkinson's disease: A hypothesis. *Can J Neurol Sci* 11(1 Suppl): 105-107

Ficalora AS, Mize RR (1989) The neurons of the substantia nigra and zona incerta which project to the cat superior colliculus are GABA immunoreactive: A double-label study using GABA immunocytochemistry and lectin retrograde transport. Neuroscience 29: 567-581

Fisher LJ, Jinnah HA, Kale LC, Higgins GA, Gage FH (1991) Survival and function of intrastriatally grafted primary fibroblasts genetically modified to produce L-dopa. *Neuron* 6: 371-380

Fisher LJ, Schinstine M, Salvaterra P, Dekker AJ, Thal L, Gage FH (1993) *In vivo* production and release of acetylcholine from primary fibroblasts genetically modified to express choline acetyl-transferase. *J Neurochem* 61: 1323-1332

Fitoussi N, Sotnik-Barkai I, Tornatore C, Herzberg U, Yadid G (1998) Dopamine turnover and metabolism in the striatum of parkinsonian rats grafted with genetically-modified human astrocytes. *Neuroscience* 85: 405-413

Flores EG (1990) Is autologous transplant of adrenal medulla into the striatum an effective therapy for Parkinson's disease? *Restorative Neurol Neurosci* 1: 182

Freed CR, Breeze RE, Rosenberg NL, Schneck SA, Kriek E, Qi J-X, Lone T, Zhang Y-B, Snyder JA, Wells, TH, Ramig LO, Thompson L, Mazziota JC, Huang SC, Grafton ST, Brooks D, Sawle G, Schroter, Ansari AA (1992) Survival of implanted fetal dopamine cells and neurologic improvement 12 to 46 months after transplantation for Parkinson's disease. *N Engl J Med* 327: 1549-1555

Freed CR, Breeze RE, Rosenberg NL, Schneck SA, Wells TH, Barrett JN, Grafton ST, Huang SC, Eidelberg D, Rottenberg DA (1990) Transplantation of human fetal dopamine cells for Parkinson's disease. *Arch Neurol* 47: 505-512

Freed WJ, Morihisa JM, Spoor E, Hoffer BJ, Olson L, Seiger Å, Wyatt RJ (1981) Transplanted adrenal chromaffin cells in rat brain reduce lesion-induced rotational behaviour. *Nature* 292: 351-352

Freed WJ, Perlow MJ, Karoum F, Seiger Å, Olson L, Hoffer BJ, Wyatt RJ (1980) Restoration of dopaminergic function by grafting of fetal rat substantia to the caudate nucleus: Long-term behavioural, biochemical, and histochemical studies. *Ann Neurol* 8: 510-519

Freeman TB, Olanow CW, Hauser RA, Nauert GM, Smith DA, Borlongan CV, Sanberg PR, Holt DA, Kordower JH, Vingerhoets FJG, Snow BJ, Calne, DB, Gauger LL (1995) Bilateral fetal nigral transplantation into the postcommissural putamen in Parkinson's disease. *Ann. Neurol* 38: 379-388

Freund TF, Bolam JP, Björklund A, Stenevi U, Dunnett SB, Powell JF, Smith AD (1985) Efferent synaptic connections of grafted dopaminergic neurons reinnervating the host neostriatum: A tyrosine hydroxylase immunocytochemical study. *J Neurosci* 5: 603-616

Galpern, WR, Burns LH, Deacon TW, Dinsmore J, Isacson O (1996) Xenotransplantation of porcine fetal ventral mesencephalon in a rat model of Parkinson's disease: Functional recovery and graft morphology. *Exp Neurol* 140: 1-13

Gasbarri A, Sulli A, Innocenzi R, Pacitti C, Brioni JD (1996) Spatial memory impairment induced by lesion of the mesohippocampal dopaminergic system in the rat. *Neuroscience* 74: 1037-1044

Gauchy C, Kemel ML, Desban M, Romo R, Glowinski J, Besson MJ (1987) The role of dopamine released from distal and proximal dendrites of nigrostriatal dopaminergic neurons in the control of GABA transmission in the thalamic nucleus ventralis medialis in the cat. *Neuroscience* 22: 935-946

Gerlach M, Youdim MBH, Riederer P (1994) Is Selegiline neuroprotective in Parkinson's disease? *J Neural Transm* 41 (Suppl): 177-188

Goetz CG, Olanow CW, Koller WC, Penn RD, Cahill D, Morantz R, Stebbins G, Tanner CM, Klawans HL, Shannon KM (1989) Multicenter study of autologous adrenal medullary transplantation to the corpus striatum in patients with advanced Parkinson's disease. *N Engl J Med* 320: 337-341

Goetz CG, Stebbins III GT, Klawans HL, Koller WC, Grossman RG, Bakay RA, Penn RD (1991) United Parkinson Foundation Neurotransplantation Registry on adrenal medullary transplants: Presurgical, and 1- and 2-year follow-up. *Neurology* 41: 1719-1722

Gould E, Woolf NJ, Butcher LL (1989) Cholinergic projections to the substantia nigra from the pedunculopontine and laterodorsal tegmental nuclei. *Neuroscience* 28: 611-623

Gorell JM, Johnson CC, Rybicki BA, Peterson EL, Kortsha GX, Brown GG, Richardson RJ (1999) Occupational exposure to manganese, copper, lead, iron, mercury and zinc and the risk of Parkinson's disease. *Neurotoxicology* 20: 239-247

Granholm AC, Mott JL, Bowenkamo K, Elken S, Henry S, Hoffer BJ, Lapchak PA, Palmer MR, Van Horne C, Gerhardt GA (1997) Glial cell line-derived neurotrophic factor improves survival of fetal ventral mesencepalic grafts to 6-hydroxydopamine lesioned striatum. *Exp Brain Res* 116: 29-38

Grasbon-Frodl EM, Nakao N, Brundin P (1996) The lazaroid U-83836E improves the survival of rat embryonic mesencephalic tissue stored at 4°C and subsequently used for cultures or intracerebral transplantation. *Brain Res Bull* 39: 341-347

Gross C, Rougier A, Guehl D, Boraud T, Julien J, Bioulac B (1997) High-frequency stimulation of the globus pallidus internalis in Parkinson's disease: A study of seven cases. *J Neurosurg* 87: 491-498

Guo Z, Du X, Iacovitti L (1998) Regulation of tyrosine hydroxylase gene expression during transdifferentiation of striatal neurons: Changes in transcription factors binding the AP-1 site. *J Neurosci* 18: 8163-8174

Guridi J, Herrero MT, Luquin MR, Guillen J, Ruberg M, Laguna J, Vila M, Javoy-Agid F, Agid Y, Hirsch E, Obeso JA (1996) Subthalamtomy in Parkinsonian monkeys. Behavioural and biochemical analysis. *Brain* 119: 1717-1727

Guttman M (1997) Double-blind comparison of pramipexole and bromocriptine treatment with placebo in advanced Parkinson's disease. International Pramipexole-Bromocriptine Study Group. *Neurology* 49: 1060-1065

Habets AM, Lopes Da Silva FH, Mollevanger WJ (1980) An olfactory input to the hippocampus of the cat: field potential analysis. *Brain Res* 182: 47-64

Hagell P, Schrag A, Piccini P, Jahanshahi M, Brown R, Rehncrona S, Widner H, Brundin P, Rothwell JC, Odin P, Wenning GK, Morrish P, Gustavii B, Björklund A, Brooks DJ, Marsden CD, Quinn NP, Lindvall O (1999) Sequential bilateral transplantation in Parkinson's disease: Effects of the second graft. *Brain* 122: 1121-1132

Halasz B, Pupp L, Uhlarik S, Tima L (1963) Growth of hyophysectomized rats bearing pituitary transplant in the hypothalamus. *Acta Physiol Hung* 23: 287-292

Halasz B, Pupp L, Uhlarik S, Tima L (1965) Further studies on the hormone secretion of the anterior pituitary transplanted into the hypophysiotrohic areas of the rat hypothalamus. *Endocrinology* 77: 343-355

Hariz MI, DeSalles A (1997) The side-effects and complications of posteroventral pallidotomy. *Acta Neurochir Suppl Wien* 68: 42-48

Hashitani T, Mizukawa K, Kumazaki M, Nishino H (1998) Dopamine metabolism in the striatum of hemiparkinsonian rats with dopaminergic grafts. *Neurosci Res* 30: 43-52

Hassani OK, Feger J (1999) Effects of intrasubthalamic injection of dopamine receptor agonists on subthalamic neurons in normal and 6-hydroxydopamine-lesioned rats: An electrophysiological and c-fos study. *Neuroscience* 92: 533-543

Hassani OK, François C, Yelnik J, Feger J (1997) Evidence for a dopaminergic innervation of the subthalamic nucleus in the rat. *Brain Res* 749: 88-94

Hassani OK, Mouroux M, Feger J (1996) Increased subthalamic neuronal activity following nigral dopaminergic lesion independent of globus pallidus inhibition. *Neuroscience* 72: 105-115

Hattori N, Kitada T, Matsumine H, Asakawa S, Yamamura Y, Yoshino H, Kobayashi T, Yokochi M, Wang M, Yoritaka A, Kondo T, Kuzuhara S, Nakamura S, Shimizu N, Mizuno Y (1998) Molecular genetic analysis of a novel Parkin gene in Japanese families with autosomal recessive juvenile parkinsonism: Evidence for variable homozygous deletions in the Parkin gene in affected individuals. *Ann Neurol* 44: 935-941

Hattori T, McGeer PL, Fibiger HC, McGeer EG (1973) On the source of GABA-containing terminals in the substantia nigra. Electron microscopic, autoradiographic and biochemical studies. *Brain Res* 54: 103-114

Hauber W (1998) Blockade of subthalamic dopamine D1 receptors elicits akinesia in rats. *Neuroreport* 9: 4115-4118

Hauser RA, Freeman TB, Snow BJ, Nauert M, Gauger L, Kordower JH, Olanow CW (1999) Long-term evaluation of fetal nigral transplantation in Parkinson's disease. *Arch Neurol* 56: 179-187

Hedreen JC (1999) Tyrosine hydroxylase-immunoreactive elements in the human globus pallidus and subthalamic nucleus. *J Comp Neurol* 409: 400-410

Hefti F, Hartikka, Schlumpf M (1985) Implantation of PC12 cells into the corpus striatum of rats with lesions of the dopaminergic nigrostriatal neurons. *Brain Res* 348: 283-288

Henderson BTH, Clough CG, Hughes RC, Hitchcock ER, Kenny BG (1991) Implantation of human fetal ventral mesencephalon to the right caudate nucleus in advanced Parkinson's disease. *Arch Neurol* 48: 822-827

Herman JP, Choulli K, Abrous N, Dulluc J, Le Moal M (1988) Effects of intra-accumbens dopaminergic grafts on behavioral deficits induced by 6-OHDA lesions of the nucleus accumbens or A10 dopaminergic neurons: A comparison. *Behav Brain Res* 29: 73-83

Hitchcock ER, Clough CG, Hughes R, Kenny B (1988) Embryos and Parkinson's disease. *Lancet* 1: 1274

Horellou P, Marlier L, Privat A, Darchen F, Scherman D, Henry J-P, Mallet J (1990a) Exogenous expression of L-dopa and dopamine in various cell lines following transfer of rat and human tyrosine hydroxylase cDNA: Grafting in an animal model of Parkinson's disease. *Prog Brain Res* 82: 23-32

Horellou P, Marlier L, Privat A, Mallet J (1990b) Behavioural effect of engineered cells that synthesize L-dopa or dopamine after grafting into the rat neostriatum. *Eur J Neurosci* 2: 116-119

Hubble JP, Busenbark KL, Wilkinson S, Pahwa R, Paulson GW, Lyons K, Koller WC (1997) Effects of deep brain stimulation based on tremor type and diagnosis. *Mov Disord* 12: 337-341

Hurlbert MS, Gianani RI, Hutt C, Freed CR, Kaddis FG (1999) Neural transplantation of hNT neurons for Huntington's disease. *Cell Transplant* 8: 143-151

Hutchinson WD, Lozano AM, Tasker RR, Lang AE, Dostrovsky JO (1997) Identification and characterization of neurons with tremor-frequency activity in human globus pallidus. *Exp Brain Res* 113: 557-563

Iacono RP, Shima F, Lonser RR, Kuniyoshi S, Maeda G, Yamada S (1995) The results, implications and physiology of posteroventral pallidotomy for patients with Parkinson's disease. *Neurosurgery* 36: 1118-1125

Iacovitti L (1991) Effects of a novel differentiation factor on the development of catecholamine traits in noncatecholamine neurons from various regions of the rat brain: Studies in tissue culture. *J Neurosci* 11: 2403-2409

Iacovitti L, Evinger MJ, Joh TH, Reis DJ (1989) A muscle-derived factor(s) induces expression of a catecholamine phenotype in neurons of cultured rat cerebral cortex. *J Neurosci* 9: 3529-3537

Iacovitti, L, Stull N (1997) Expression of tyrosine hydroxylase in newly differentiated neurons from a human cell line (hNT). *Neuroreport* 8: 1471-1474

Imaoka T, Date I, Ohmoto T, Nagatsu T (1998) Significant behavioral recovery in a Parkinson's disease model by direct intracerebral gene transfer using continuous injection of a plasmid DNA-liposome complex. *Hum Gene Ther* 9: 1093-1102

Isacson O, Breakefield XO (1997) Benefits and risks of hosting animal cells in the human brain. *Nat Med* 3: 964-969

Isacson O, Deacon TW (1996) Specific axon guidance factors persist in the adult brain as demonstrated by pig neuroblasts transplanted to the rat. *Neuroscience* 75: 827-837

Isacson O, Deacon TW, Pakzaban P, Galpern WR, Dinsmore J, Burns LH (1995) Transplanted xenogeneic neural cells in neurodegenerative disease models exhibit remarkable axonal target specificity and distinct growth patterns of glial and axonal fibres. *Nat Med* 1: 1189-1194

Ishida A, Yamashiro K, Mukawa J, Hasegawa M (1996) Regulation of L-DOPA production by genetically modified primary fibroblasts transfected with retrovirus vector system. *Cell Transplant* 5 (Suppl 1): S5-S7

Ishida Y, Hashiguchi H, Yamamoto R, Hashitani T, Ikeda T, Nishino H (1991) Effect of intra-amygdala dopaminergic grafts on methamphetamine-induced locomotor activity, extracellular dopamine and dopamine metabolite overflow: A comparison with the effect of intra-accumbens grafts. *Brain Res* 549: 342-345

Jackson EA, Kelly PH (1983a) Nigral dopaminergic mechanisms in drug-induced circling. *Brain Res Bull* 11: 605-611

Jackson EA, Kelly PH (1983b) Role of nigral dopamine in amphetamine-induced locomotor activity. *Brain Res* 278: 366-369

Jankovic J, Cardoso F, Grossman RG, Hamilton WJ (1995) Outcome after stereotactic thalamotomy for Parkinsonian, essential and other types of tremor. *Neurosurgery* 37: 680-687

Jiang N, Jiang C, Tang Z, et al. (1987) Human foetal brain transplant trials in the treatment of Parkinsonism. *Acta Acad Medicin Shanghai* 14: 77.

Jin BK, Iacovitti L (1995) Dopamine differentiation factors produce partial motor recovery in 6-hydroxydopamine lesioned rats. *Neurobiol Dis* 2: 1-12

Jin BK, Iacovitti L (1996) Dopamine differentiation factors increase striatal dopaminergic function in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned mice. *J Neurosci Res* 43: 331-334

Johnson RL, Laufer E, Riddle RD, Tabin C (1994) Ectopic expression of Sonic hedgehog alters dorsal-ventral patterning of somites. *Cell* 79: 1165-1173

Ju WYH, Holland DP, Tatton WG (1994) (-)-deprenyl alters the time course of death of axotomized facial motoneurns and the hypertrophy of neighbouring astrocytes in immature rats. *Exp Neurol* 126: 233-246

Kaneko T, Mizuno M (1988) Immunohistochemical study of glutaminase-containing neurons in the cerebral cortex and thalamus of the rat. *J Comp Neurol* 267: 590-602

Karoum F, Chuang L-W, Eisler T, Calne DB, Liebowitz MR, Quitkin FM, Klein DF, Wyatt RJ (1982) Metabolism of (-) deprenyl to amphetamine and methamphetamine may be responsible for deprenyl's therapeutic benefit: A biochemical assessment. *Neurology* 32: 503-509

Kelly PJ, Ahlskog JE, Van Heerden JA, Carmichael SW, Stoddard SL, Bell GN (1989) Adrenal medullary autograft transplantation into the striatum of patients with Parkinson's disease. *Mayo Clin Proc* 64: 282-290

Kemel ML, Desban M, Gauchy C, Glowinski J, Besson MJ (1988) Topographical organization of efferent projections from the cat substantia nigra pars reticulata. *Brain Res* 455: 307-323

Kilpatrick IC, Starr MS, Fletcher A, James TA, MacLeod NK (1980) Evidence for a GABAergic nigrothalamic pathway in the rat. I. Behavioural and biochemical studies. *Exp Brain Res* 40: 45-54

Kishore A, Turnbull M, Snow BJ, de la Fuente-Fernandez R, Schulzer M, Mak E, Yardley S, Calne DB (1997) Efficacy, stability and predictors of outcome of pallidotomy for Parkinson's disease. Six-month follow-up and additional 1-year observations. *Brain* 120: 729-737

Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, Yokochi M, Mizuno Y, Shimizu N (1998) Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* 392: 605-608

Kitani K, Miyasaka K, Kanai S, Carrillo MC, Ivy GO (1996) Upregulation of antioxidant activities by deprenyl. Implications for life-span extension. *Ann NY Acad Sci* 786: 391-409

Kleppner SR, Robinson KA, Trojanowski JQ, Lee VM-Y (1995) Transplanted human neurons derived from a teratocarcinoma cell line (NTera-2) mature, integrate and survive for over 1 year in the nude mouse brain. *J Comp Neurol* 357: 618-632

Kofler P, Wiesenhofer B, Rehrl C, Baier G, Stockhammer G, Humpel C (1998) Liposome-mediated gene transfer into established CNS lines, primary glial cells, and *in vivo*. *Cell Transplant* 7: 175-185

Koller W, Pahwa R, Buesenbark K, Hubble JP, Wilkinson S, Lang AE, Tuite P, Sime E, Lozano AM, Hauser RA, Malapira T, Smith DA, Tarsy D, Miyawaki E, Norregaard T, Kormos T, Olanow CW (1997) High-frequency unilateral thalamic stimulation in the treatment of essential tremor. *Ann Neurol* 42: 292-299

Kondoh T, Pundt LL, Blount JP, Conrad JA, Low WC (1996) Transplantation of human fetal tissue from spontaneous abortions to a rodent model of Parkinson's disease. *Cell Transplant* 5: 69-75

Konobu T, Sessler F, Luo LY, Lehmann J (1998) The hNT human neuronal cell line survives and migrates into rat retina. *Cell Transplant* 7: 549-558

Kopin IJ (1994) Neurotransmitters and disorders of the basal ganglia. In: *Basic Neurochemistry* (5th ed) (Siegel GJ, Agranoff BW, Albers RW, Molinoff PB, eds), New York, Raven Press, Ltd., 899-918

Kopyov O, Jacques D, Duma C, Buckwalter G, Kopyov A, Lieberman A, Copcutt B (1997a) Microelectrode-guided posteroventral medial radiofrequency pallidotomy for Parkinson's disease. *J Neurosurg* 87: 52-59

Kopyov OV, Jacques D'S', Lieberman A, Duma CM, Rogers RL (1997b) Outcome following intrastriatal fetal mesencephalic grafts for Parkinson's patients is directly related to volume of grafted tissue. *Exp Neurol* 146: 536-545

Korczyn AD, Brooks DJ, Brunt ER, Poewe WH, Rascol O, Stocchi F (1998) Ropinirole versus bromocriptine in the treatment of early Parkinson's disease: A 6-month interim report of a 3-year study. 053 Study Group. *Mov Disord* 13: 46-51

Kordower JH, Freeman TB, Snow BJ, Vingerhoets FJG, Mufson EJ, Sanberg PR, Hasuer RA, Smith DA, Nauert GM, Perl DP, Olanow CW (1995) Neuropathological evidence of graft survival and striatal reinnervation after the transplantation of fetal mesencephalic tissue in a patient with Parkinson's disease. *N Engl J Med* 332: 1118-1124

Kordower JH, Goetz CG, Freeman TB, Olanow CW (1997) Dopaminergic transplants in patients with Parkinson's disease: Neuroanatomical correlates of clinical recovery. *Exp Neurol* 144: 41-46

Kordower JH, Rosenstein JM, Collier TJ, Burke MA, Chen E-Y, Li JM, Martel L, Level AE, Mufson EJ, Freeman TB, Olanow CW (1996) Functional fetal nigral grafts in a patient with Parkinson's disease: Chemoanatomic, ultrastructural, and metabolic studies. *J Comp Neurol* 370: 203-230

Krack P, Benazzouz A, Pollak P, Limousin P, Piallat B, Hoffmann D, Xie J, Benabid AL (1998a) Treatment of tremor in Parkinson's disease by subthalamic nucleus stimulation. *Mov Disord* 13: 907-914

Krack P, Limousin P, Benabid AL, Pollak P (1997a) Chronic stimulation of subthalamic nucleus improves levodopa-induced dyskinesias in Parkinson's disease. *Lancet* 350: 1676

Krack P, Pollak P, Limousin P, Benazzouz A, Benabid AL (1997b) Stimulation of subthalamic nucleus alleviates tremor in Parkinson's disease. *Lancet* 350: 1675

Krack P, Pollak P, Limousin P, Hoffmann D, Xie J, Benazzouz A & Benabid AL (1998b) Subthalamic nucleus or internal pallidal stimulation in young onset Parkinson's disease. *Brain* 121: 451-457

Krauss JK, Desaloms M, Lai EC, King DE, Jankovic J, Grossman RG (1997) Microelectrode-guided posteroventral pallidotomy for treatment of Parkinson's disease: Postoperative magnetic resonance imaging analysis. *J Neurosurg* 87: 358-367

Kumar R, Lozano AM, Kim YJ, Hutchinson WD, Sime E, Halket E, Lang AE (1998a) Double-blind evaluation of subthalamic nucleus deep brain stimulation in Parkinson's disease. *Neurology* 51: 850-855

Kumar R, Lozano AM, Sime E, Halket E, Lang AE (1999) Comparatve effects of unilateral and bilateral subthalamic nucleus deep brain stimulation. *Neurology* 53: 561-566

Kumar R, Lozano AM, Montgomery E, Lang AE (1998b) Pallidotomy and deep brain stimulation of the pallidum and subthalamic nucleus in Parkinson's disease. *Mov Disord* 13 (Suppl 1): 73-82

Kunzle H (1975) Bilateral projections from precentral motor cortex to the putamen and other parts of the basal ganglia. An autoradiographic study in *Macaca fascicularis*. *Brain Res* 88: 195-209

Kunzle H (1977) Projections from the primary somatosensory cortex to basal ganglia and thalamus in the monkey. *Exp Brain Res* 30: 481-492

Kunzle H, Akert K (1977) Efferent connections of cortical, area 8 (frontal eye fields) in *Macaca fascicularis*. A reinvestigation using the autoradiographic technique. *J Comp Neurol* 173: 153-164

Laitinen LV, Bergenheim AT, Hariz MI (1992) Leksell's posteroventral pallidotomy in the treatment of Parkinson's disease. *J Neurosurg* 76: 53-61

Lang AE, Lozano AM, Montgomery E, Duff J, Tasker R, Hutchinson W (1997) Posteroventral pallidotomy in advanced Parkinson's disease. *N Engl J Med* 337: 1036-1042

Langston JW, Ballard P, Tetrud JW, Irwin I (1983) Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science* 219: 979-980

Lavoie B, Parent A (1994) Pedunculopontine nucleus in the squirrel monkey: Cholinergic and glutamatergic projections to the substantia nigra. *J Comp Neurol* 344: 232-241

Lavoie B, Smith Y, Parent A (1989) Dopaminergic innervation of the basal ganglia in the squirrel monkey as revealed by tyrosine hydroxylase immunohistochemistry. *J Comp Neurol* 289: 36-52

Lee VM-Y, Andrews PW (1986) Differentiation of NTERA-2 clonal human embryonal carcinoma cells into neurons involved the induction of all three neurofilament proteins. *J Neurosci* 6: 514-521

Leff SE, Rendahl KG, Spratt SK, Kang UJ, Mandel RJ (1998) *In vivo* L-DOPA production by genetically modified primary rat fibroblast or 9L gliosarcoma cell grafts via coexpression of GTPcyclohydrolase I with tyrosine hydroxylase. *Exp Neurol* 151: 249-264

Le Gros Clark WE (1940) Neuronal differentiation in implanted foetal cortical tissue. *J Neural Psychiatry* 3: 263-284

Lenard L, Hahn Z (1982) Amygdalar noradrenergic and dopaminergic mechanisms in the regulation of hunger and thirst-motivated behavior. *Brain Res* 233: 115-132

Lenz, FA, Normand SI, Kwan HC, Andrews D, Rowland LH, Jones MW, Seike M, Lin YC, Tasker RR, Dostrovsky JO, Lenz YE (1995) Statistical prediction of the optimal site for thalamotomy in Parkinsonian tremor. *Mov Disord* 10: 318-328

Leroy E, Anastasopoulos D, Konitsiotis S, Lavedan C, Polymeropoulos MH (1998) Deletions in the Parkin gene and genetic heterogeneity in a Greek family with early onset Parkinson's disease. *Hum Genet* 103: 424-427

Levivier M, Dethy S, Rodesch F, Peschanski M, Vandesteene A, David P, Wikler D, Goldman S, Claes T, Biver F, Liesnard C, Goldman M, Hildebrand J, Brotchi J (1997) Intracerebral transplantation of fetal ventral mesencephalon for patients with advanced Parkinson's disease. Methodology and 6-month to 1-year follow-up in 3 patients. *Stereotact Funct Neurosurg* 69: 99-111

Levivier M, Przedborski S, Bencsics C, Kang UJ (1995) Intrastriatal implantation of fibroblasts genetically engineered to produce brain-derived neurotrophic factor prevents degeneration of dopaminergic neurons in a rat model of Parkinson's disease. *J Neurosci* 15: 7810-7820

Levy R, Hazrati L-N, Herrero MT Vila M, Hassani O-K, Mouroux M, Ruberg M, Asensi H, Agid Y, Feger J, Obeso JA, Parent A, Hirsch EC (1997) Re-evaluation of the function of the basal ganglia in normal and Parkinsonian states. *Neuroscience* 76: 335-343

LeWitt PA, the Parkinson Study Group (1991) Deprenyl's effect at slowing progression of Parkinsonian disability: The DATATOP study. *Acta Neurol Scand* 136 (suppl.): 79-86

Lieberman A, Fazzini E (1991) Experience with selegiline and levodopa in advanced Parkinson's disease. *Acta Neurol Scand Suppl* 136: 66-69

Lieberman A, Olanow CW, Sethi K, Swanson P, Waters CH, Fahn S, Hurtig H, Yahr M (1998) A multicenter trial of ropinirole as adjunct treatment for Parkinson's disease. Ropinirole Study Group. *Neurology* 51: 1057-62

Lieberman A, Ranhosky A, Korts D (1997) Clinical evaluation of pramipexole in advanced Parkinson's disease: Results of a double-blind, placebo-controlled, parallel-group study. *Neurology* 49: 162-168

Limousin P, Greene J, Pollak P, Rothwell JC, Benabid AL, Frackowiak R (1997) Changes in cerebral activity pattern due to subthalamic nucleus or internal pallidum stimulation in Parkinson's disease. *Ann Neurol* 42: 283-291

Limousin P, Krack P, Pollak P, Benazzouz A, Ardouin C, Hoffmann D, Benabid AL (1998) Electrical stimulation of the subthalamic nucleus in advanced Parkinson's disease. *N Engl J Med* 339: 1105-1111

Lin JJ, Yueh KC, Chang DC, Lin SZ (1999) Absence of G209A and G88C mutations in the α-synuclein gene of Parkinson's disease in a Chinese population. *Eur Neurol* 42: 217-220

Lindvall O (1998) An update on fetal transplantation: The Swedish experience. *Mov Disord* 13 (Suppl 1): 83-87

Lindvall O, Backlund EO, Farde L, Sedvall G, Freedman R, Hoffer B, Nobin A, Seiger Å, Olson L (1987) Transplantation in Parkinson's disease: Two cases of adrenal medullary grafts to putamen. *Ann Neurol* 22: 457-468

Lindvall O, Brundin P, Widner H, Rehncrona S, Gustavii B, Frackowiak R, Leenders KL, Sawle G, Rothwell JC, Marsden CD, Björklund A (1990) Grafts of fetal dopamine neurons survive and improve motor function in Parkinson's disease. *Science* 247: 574-577

Lindvall O, Rehncrona S, Brundin P, Gustavii B, Åstedt B, Widner H, Lindholm T, Björklund A, Leenders KL, Rothwell JC, Frackowiak RC, Marsden CD, Johnels B, Steg G, Freedman R, Hoffer BJ, Seiger Å, Bygdeman M, Strömberg I, Olson L (1989) Human fetal dopamine neurons grafted into the striatum of two patients with severe Parkinson's disease. A detailed account of methodology and a 6-month follow-up. *Arch Neurol* 46: 615-631

Lindvall O, Rehncrona S, Gustavii B, Brundin P, Åstedt B, Widner H, Lindholm T, Björklund A, Leenders KL, Rothwell JC, Frackowiak RC, Marsden CD, Johnels B, Steg G, Freedman R, Hoffer BJ, Seiger Å, Strömberg I, Bygdeman M, Olson L (1988) Fetal dopamine-rich mesencephalic grafts in Parkinson's disease. *Lancet* 2: 1483

Lindvall O, Sawle, Widner H, Rothwell JC, Björklund, Brooks D, Brundin P, Frackowiak R, Marsden CD, Odin P, Rehncrona S (1994) Evidence for long-term survival and function of dopaminergic grafts in progressive Parkinson's disease. *Ann Neurol* 35: 172-180

Lindvall O, Widner H, Rehncrona S, Brundin P, Odin P, Gustavii B, Frackowiak R, Leenders KL, Sawle G, Rothwell JC, Björklund A, Marsden CD (1992) Transplantation of fetal dopamine neurons in Parkinson's disease: One-year clinical and neurophysiological observations in two patients with putaminal implants. *Ann Neurol* 31: 155-165

Ljungberg MC, Stern G, Wilkin GP (1999) Survival of genetically engineered, adult-derived rat astrocytes grafted into the 6-hydroxydopamine lesioned adult rat striatum. *Brain Res* 816: 29-37

Loopujit LD, Van Der Rooy (1985) Organization of the striatum: Collateralization of its efferent axons. *Brain Res* 348: 86-99

López-Lozano JJ, Bravo G, Brera B, Dargallo J, Salmeán J, Uría J, Insausti J, Millán I (1995) Long-term follow-up in 10 Parkinson's disease patients subjected to fetal brain grafting into a cavity in the caudate nucleus: The Clinica Puerta de Hierro experience. CPH Neural Transplantation Group. *Transplant Proc* 27: 1395-1400

López-Lozano JJ, Bravo G, Brera B, Millán I, Dargallo J, Salmeán J, Uría J, Insausti J, the Clínica Puerta de Hierro Neural Transplantation Group (1997) Long-term improvement in patients with severe Parkinson's disease after implantation of fetal ventral mesencephalic tissue in a cavity of the caudate nucleus: 5-year follow up in 10 patients. *J Neurosurg* 86: 931-942

Lozano AM, Lang AE, Galvez-Jimenez N, Miyasaki J, Hutchinson WD, Dostrovsky JO (1995) Effect of GPi pallidotomy on motor function in Parkinson's disease. *Lancet* 346: 1383-1387

Lund R, Hauscha S (1976) Transplanted neural tissue develops connections with host rat brain. *Science* 193: 582-584

Lundberg C, Björklund A (1996) Host regulation of glial markers in intrastriatal grafts of conditionally immortalized neural stem cell lines. *Neuroreport* 7: 847-852

Lundberg C, Horellou P, Mallet J, Björklund A (1996) Generation of DOPA-producing astrocytes by retroviral transduction of the human tyrosine hydroxylase gene: *In vitro* characterization and *in vivo* effects in the rat Parkinson model. *Exp Neurol* 139: 39-53

MacLeod NK, James TA, Kilpatrick IC, Starr MS (1980) Evidence for a GABAergic nigrothalamic pathway in the rat. II. Electrophysiological studies. *Exp Brain Res* 40: 55-61

Madrazo I, Drucker-Colín R, Díaz V, Martinez-Mata J, Torres C, Becerril JJ (1987) Open microsurgical autograft of adrenal medulla to the right caudate nucleus in two patients with intractable Parkinson's disease. *N Engl J Med* 316: 831-834

Madrazo I, Franco-Bourland R, Ostrosky-Solis F, Aguilera M, Cuevas C, Alvarez F, Magallon E, Zamorano C, Morelos A (1990a) Neural transplantation (auto-adrenal, fetal nigral and fetal adrenal) in Parkinson's disease: The Mexican experience. *Prog Brain Res* 82: 593-602

Madrazo I, Franco-Bourland R, Ostrosky-Solis F, Aguilera M, Cuevas C, Zamorano C, Morelos A, Magallon E, Guizar-Sahagun G (1990b) Fetal homotransplants (ventral mesencephalon and adrenal tissue) to the striatum of Parkinsonian subjects. *Arch Neurol* 47: 1281-1285

Madrazo I, Leon V, Torres C, Aguilera MC, Varela G, Alvarez F, Fraga A, Drucker-Colín R, Ostrosky F, Skurovich M, et al (1988) Transplantation of fetal substantia nigra and adrenal medulla to the caudate nucleus in two patients with Parkinson's disease. *N Engl J Med* 318: 51

Manaster JS, Feuerman T, Reynolds CP, Markham CH (1992) Transplantation of human neuroblastoma cells, catecholaminergic and non-catecholaminergic: Effects on rotational behavior in Parkinson's rat model. *J Neural Transplant Plast* 3: 139-150

Mantione JR, Kleppner SR, Miyazono M, Wertkin AM, Lee VM-Y, Trojanowski (1995) Human neurons that constitutively secrete Aβ do not induce Alzheimer's disease pathology following transplantation and long-term survival in the rodent brain. *Brain Res* 671: 333-337

Marder K, Logroscino G, Alfaro B, Mejia H, Halim A, Louis E, Cote L, Mayeux R (1998) Environmental risk factors for Parkinson's disease in an urban multiethnic community. *Neurology* 50: 279-281

Marsden CD, Parkes JD (1977) Success and problems of long-term levodopa therapy in Parkinson's disease. *Lancet* 1: 345-349

Matsumoto K, Shichijo F, Taukami T (1984) Long-term follow-up review of cases of Parkinson's disease after unilateral of bilateral thalamotomy. *J Neurosurg* 60: 1033-1044

May RM (1949) Connexions entre de cellules cerebrales et des muscles de la cause dans leur greffe blephoplastique intraoculaire simultenee chez la souris. *Anat Microsc Morphol Exp* 38: 145

Mayer E, Fawcett JW, Dunnett SB (1993) Basic fibroblast growth factor promotes the survival of embryonic ventral mesencephalic dopaminergic neurons--II. Effects on nigral transplants *in vivo*. *Neuroscience* 56: 389-398

Mehta V, Hong M, Spears J, Mendez I (1998) Enhancement of graft survival and sensorimotor behavioural recovery in rats undergoing transplantation with dopaminergic cells exposed to glial cell line-derived neurotrophic factor. *J Neurosurg* 88: 1088-1095

Mehta V, Spears J, Mendez I (1997) Neural transplantation in Parkinson's disease. *Can J Neurol Sci* 24: 292-301

Mena MA, Pardo B, Casarejos MJ, Fahn S, De Yebenes JG (1992) Neurotoxicity of levodopa on catecholamine-rich neurons. *Mov Disord* 7: 23-31

Mendez I, Elisevich K, Flumerfelt B (1991) Dopaminergic innervation of substance P-containing striatal neurons by fetal nigral grafts: An ultrastructural double-labeling immunocytochemical study. *J Comp Neurol* 308: 66-78

Mendez I, Elisevich K, Naus C, Flumerfelt B (1992) Restoration of nigrostriatal synaptic circuitry, striatal mRNA expression, and motor symmetry following embryonic substantia nigra grafts. *Clin Neurosurg* 38: 180-209

Mendez I, Hong M (1997) Reconstruction of the striato-nigro-striatal circuitry by simultaneous double dopaminergic grafts: A tracer study using fluorogold and horseradish peroxidase. *Brain Res* 778: 194-205

Mendez I, Naus CC, Elisevich K, Flumerfelt BA (1993) Normalization of striatal proenkephalin and preprotachykinin expression by fetal substantia nigra grafts. *Exp Neurol* 119: 1-10

Mendez I, Sadi D, Hong M (1996) Reconstruction of the nigrostriatal pathway by simultaneous intrastriatal and intranigral dopaminergic transplants. *J Neurosci* 16: 7216-7227

Miyazono M, Lee VM-Y Trojanowski JQ (1995) Proliferation, cell death, and neuronal differentiation in transplanted human embryonal carcinoma (NTera2) cells depend on the graft site in nude and severe combined immunodeficient mice. *Lab Invest* 73: 273-283

Miyazono M, Nowell PC, Finan JL, Lee VM-Y, Trojanowski JQ (1996) Long-term integration and neuronal differentiation of human embryonal carcinoma cells (NTera-2) transplanted into the caudoputamen of nude mice. *J Comp Neurol* 376: 603-613

Moore RY, Bloom FE (1978) Central catecholamine neuron systems: Anatomy and physiology of the dopamine systems. *Ann Rev Neurosci* 1: 129-169

Moro E, Scerrati M, Romito LM, Roselli R, Tonali P, Albanese A (1999) Chronic subthalamic nucleus stimulation reduces medication requirements in Parkinson's disease. *Neurology* 53: 85-90

Morrow BA, Elsworth JD, Rasmusson AM, Roth RH (1999) The role of mesoprefrontal dopamine neurons in the acquisition and expression of conditioned fear in the rat. *Neuroscience* 92: 553-564

Moukhles H, Forni C, Nieoullon A, Daszuta A (1994) Regulation of dopamine levels in intrastriatal grafts of fetal ventral mesencephalic cell suspension: An *in vivo* voltammetric approach. *Exp Brain Res* 102: 10-20

Muir JK, Raghupathi R, Saatman KE, Wilson CA, VM Lee, Trojanowski JQ, Philips MF, Sanberg PR (1999) Terminally differentiated human neurons survive and integrate following transplantation into the traumatically injured rat brain. *J Neurotrauma* 16: 403-414

Myllylä VV, Sontaniemi KA, Vuorinen JA, Heinnonen EH (1991) Selegiline as a primary treatment of Parkinson's disease. *Acta Neurol Scand Suppl* 136: 70-72

Myllylä VV, Sontaniemi KA, Vuorinen JA, Heinnonen EH (1992) Selegiline as initial treatment in *de novo* Parkinsonian patients. *Neurology* 42: 339-343

Mytilineou C, Radcliffe P, Leonardi EK, Werner P, Olanow CW (1997) L-deprenyl protects mesencephalic dopamine neurons from glutamate receptor-mediated toxicity *in vitro*. *J Neurochem* 68: 33-39

Nakao N, Frodl EM, Duan W-M, Widner H, Brundin P (1994) Lazaroids improve the survival of grafted rat embryonic dopamine neurons. *Proc Natl Acad Sci USA* 91: 12408-12412

Nakao N, Ogura M, Nakai K, Itakura T (1998) Intrastriatal mesencephalic grafts affect neuronal activity in basal ganglia nuclei and their target structures in a rat model of Parkinson's disease. *J Neurosci* 18: 1806-1817

Nambu A, Yoshida S, Jinnai K (1988) Projection to motor cortex of thalamic neurons with pallidal input in the monkey. *Exp Brain Res* 71: 658-662

Nankova B, Hiremagalur B, Menezes A, Zeman R, Sabban E (1996) Promoter elements and second messenger pathways involved in transcriptional activation of tyrosine hydroxylase by ionomycin. *Brain Res Mol Brain Res* 35: 164-172

Nikkhah G, Bentlage C, Cunningham MG, Björklund A (1994a) Intranigral fetal dopamine grafts induce behavioural compensation in the rat Parkinson model. *J Neurosci* 14: 3449-3461

Nikkhah G, Cunningham MG, Jödicke A, Knappe U, Björklund A (1994b) Improved graft survival and striatal reinnervation by microtransplantation of fetal nigral cell suspensions in the rat Parkinson model. *Brain Res* 633: 133-143

Nikkhah G, Cunningham MG, McKay R, Björklund A (1995b) Dopaminergic transplants into the substantia nigra of neonatal rats with bilateral 6-OHDA lesions. II. Transplant-induced behavioural recovery. *J Neurosci* 15: 3562-3570

Nikkhah G, Duan W-M, Knappe U, Björklund (1993) Restoration of complex sensorimotor behaviour and skilled forelimb use by a modified nigral cell suspension transplantation approach in the rat Parkinson model. *Neuroscience* 56: 33-43

Nishino H, Hashitani T, Kumazaki M, Sato H, Furuyama F, Isobe Y, Watari N, Kanai M, Shiosaka S (1990) Long-term survival of grafted cells, dopamine synthesis/release, synaptic connections, and functional recovery after transplantation of fetal nigral cells in rats with unilateral 6-OHDA lesions in the nigrostriatal dopamine pathway. *Brain Res* 534: 83-93

Nishiyama K, Mizuno T, Sakuta M, Kurisaki H (1993) Chronic dementia in Parkinson's disease treated by anticholinergic agents. Neuropsychological and neuroradiological examination. *Adv Neurol* 60: 479-483

O'Connor WT (1998) Functional neuroanatomy of the basal ganglia as studied by dual-probe microdialysis. *Nucl Med Biol* 25: 743-746

Olanow CW, Kordower JH, Freeman TB (1996) Fetal nigral transplantation as a therapy for Parkinson's disease. *Trends Neurosci* 19: 102-109

Olazabal UE, Moore JK (1989) Nigrotectal projection to the inferior colliculus: Horseradish peroxidase transport and tyrosine hydroxylase immunohistochemical studies in rats, cats, and bats. *J Comp Neurol* 282: 98-118

Olsson M, Bentlage C, Wictorin K, Campbell K, Björklund A (1997) Extensive migration and target innervation by striatal precursors after grafting into the neonatal striatum. *Neuroscience* 79: 57-78

Olsson M, Nikkhah G, Bentlage C, Björklund (1995) Forelimb akinesia in the rat Parkinson model: Differential effects of dopamine agonists and nigral transplants as assessed by a new stepping test. *J Neurosci* 15: 3863-3875

Ondo W, Hunter C, Almaguer M, Gancher S, Jankovic J (1999) Efficacy and tolerability of a novel sublingual apomorphine preparation in patients with fluctuating Parkinson's disease. *Clin Neuropharmacol* 1999 22: 1-4

Othberg A, Keep M, Brundin P, Lindvall O (1997) Tirilazad mesylate improves survival of rat and human embryonic mesencephalic neurons *in vitro*. *Exp Neurol* 47: 498-502

Othberg AI, Willing AE, Cameron DF, Anton A, Saporta S, Freeman TB, Sanberg PR (1998) Trophic effects of porcine Sertoli cells on rat and human ventral mesencephalic cells and hNT neurons *in vitro*. *Cell Transplant* 7: 157-164

Pahwa R, Wilkonson S, Smith D, Lyons K, Miyawaki E, Koller WC (1997) High-frequency stimulation of the globus pallidus for the treatment of Parkinson's disease. *Neurology* 49: 249-253

Palmer, TD, Rosman GJ, Osborne, WRA, Miller AD (1991) Genetically modified skin fibroblasts persist long after transplantation but gradually inactivate introduced genes. *Proc Natl Acad Sci USA* 88: 1330-1334

Papadimitriou A, Veletza V, Hadjigeorgiou GM, Patrikiou A, Hirano M, Anastasopoulos I, (1999) Mutated alpha-synuclein gene in two Greek kindreds with familial PD: Incomplete penetrance? *Neurology* 52: 651-654

Pardo B, Mena MA, Casarejos MJ, Paino CL, De Yebenes JG (1995) Toxic effects of L-DOPA on mesencephalic cell cultures: Protection with antioxidants. *Brain Res* 682: 133-143

Parkinson Study Group (1989) Effect of deprenyl on the progression of disability in early Parkinson's disease. *N Engl J Med* 321: 1364-1371

Parkinson Study Group (1993) Effects of tocopherol and deprenyl on the progression of disability in early Parkinson's disease. *N Engl J Med* 328: 176-183

Parsian A, Racette B, Zhang ZH, Chakraverty S, Rundle M, Goate A, Perlmutter JS (1998) Mutation, sequence analysis, and association studies of alpha-synuclein in Parkinson's disease. *Neurology* 51: 1757-1759

Perlow MJ, Freed WJ, Hoffer BJ, Seiger Å, Olson L, Wyatt RJ (1979) Brain grafts reduce motor abnormalities produced by destruction of nigro-striatal dopamine system. *Science* 204: 643-647

Peschanski M, Defer G, N'Guyen JP, Ricolfi F, Monfort JC, Remy P, Geny C, Samson Y, Hantraye P, Jeny R, Gaston A, Kéravel Y, Degos JD, Cesaro P (1994) Bilateral motor improvement and alteration of L-dopa effect in two patients with Parkinson's disease following intrastriatal transplantation of foetal ventral mesencephalic tissue in a patient with Parkinson's disease. *Brain* 117: 487-499

Pezzoli G, Martignoni E, Pacchetti C, Angeleri V, Lamberti P, Muratorio A, Bonuccelli U, De Mari M, Foschi N, Cossutta E, Nicoletti F, Giammona G, Canesi M, Scarlato G, Caraceni T, Moscarelli E (1995) A crossover, controlled study comparing pergolide with bromocriptine as an adjunct to levodopa for the treatment of Parkinson's disease. *Neurology* 45 (Suppl 3): S22-S27

Pfann KD, Penn RD, Shannon KM, Shapiro MB, Corcos DM (1996) Effect of stimulation in the ventral intermediate nucleus of the thalamus on limb control in Parkinson's disease: A case study. *Mov Disord* 11: 311-316

Philips MF, Muir JK, Saatman KE, Raghupathi R, Lee VM, Trojanowski JQ & McIntosh TK (1999) Survival and integration of transplanted postmitotic human neurons following experimental brain injury immunocompetent rats. *J Neurosurg* 90: 116-124

Phillips JM, Latimer MP, Gupta S, Winn P, Brown VJ (1998) Excitotoxic lesions of the subthalamic nucleus ameliorate asymmetry induced by striatal dopamine depletion in the rat. *Behav Brain Res* 90: 73-77

Pietz K, Hagell P, Odin P (1998) Subcutaneous apomorphine in late stage Parkinson's disease: A long term follow up. *J Neurol Neurosurg Psychiatry* 65: 709-716

Pleasure SJ, Lee VM-Y (1993) NTera 2 cells: A human cell line which displays characteristics expected of a human committed neuronal progenitor cell. *J Neurosci Res* 15: 585-602

Pleasure SJ, Page C, Lee VM-Y (1992) Pure, postmitotic, human neurons derived from NTera 2 cells provide a system for expressing exogenous proteins in terminally differentiated neurons. *J Neurosci* 12: 1802-1815

Polymeropoulos, MH, Lavedan C, Leroy E; Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, Stenroos ES, Chandrasekharappa S, Athanassiadou A, Papapetropoulos T, Johnson WG, Lazzarini AM, Duvoisin RC, Di Iorio G, Golbe LI, Nussbaum RL (1997) Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science* 276: 2045-2047

Pondal M, Del Ser T, Bermejo F (1996) Anticholinergic therapy and dementia in patients with Parkinson's disease. *J Neurol* 243: 543-546

Presse F, Cardona B, Borsu L, Nahon JL (1997) Lithium increases melanin-concentrating hormone mRNA stability and inhibits tyrosine hydroxylase gene expression in PC12 cells. *Brain Res Mol Brain Res* 52: 270-283

Rajakumar N, Elisevich K, Flumerfelt BA (1994) The pallidostriatal projection in the rat: A recurrent inhibitory loop? *Brain Res* 651: 332-336

Rascol O, Sabatini U, Choller F (1992) Supplementary and primary sensory motor area activity in Parkinson's disease. Regional cerebral blood flow changes during finger movements. *Arch Neurol* 49: 144-148

Rassnick S, Stinus L, Koob GF (1993) The effects of 6-hydroxydopamine lesions of the nucleus accumbens and the mesolimbic dopamine system on oral self-administration of ethanol in the rat. *Brain Res* 623: 16-24

Raymon HK, Thode S, Gage FH (1997) Application of *ex vivo* gene therapy in the treatment of Parkinson's disease. *Exp Neurol* 144: 82-91

Redmond DE Jr, Sladek JR Jr, Roth RH, Collier TJ, Elsworth JD, Deutch AY, Haber S (1986) Fetal neuronal grafts in monkeys given methyphenyltetrahydropyridine. *Lancet* 1: 1125-1127

Reum T, Morgenstern R (1994) Fetal mesencephalic grafts influence the dopamine release in the non-lesioned striatum of 6-OHDA-lesioned rats: A behavioral and *in vivo* voltammetric study. *Neurosci Lett* 173: 172-176

Reynolds BA, Weiss S (1992) Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science* 255: 1707-1710

Ribak CE, Vaughn JE, Saito K, Barber RP, Roberts E (1976) Immunocytochemical localization of glutamate decarboxylase in rat substantia nigra. Brain Res 116: 287-298

Rioux L, Gaudin DP, Bui LK, Gregoire L, Di Paolo T, Bédard PJ (1991) Correlation of functional recovery after a 6-hydroxydopamine lesion with survival of grafted fetal neurons and release of dopamine in the striatum of the rat. *Neuroscience* 40: 123-131

Robertson GS, Fine A, Robertson HA (1991) Dopaminergic grafts in the striatum reduce D_1 but not D_2 receptor-mediated rotation in 6-OHDA lesioned rats. *Brain Res* 539: 304-311

Robertson GS, Robertson HA (1989) Evidence that L-Dopa-induced rotational behaviour is dependent on both striatal and nigral mechanisms. *J Neurosci* 9: 3326-3331

Robertson GS, Vincent SR, Fibiger HC (1992) D1 and D2 dopamine receptors differentially regulate c-fos expression in striatonigral and striatopallidal neurons. *Neuroscience* 49: 285-96

Robertson HA (1992a) Dopamine receptor interactions: Some implications for the treatment of Parkinson's disease. *Trends Neurosci* 15: 201-206

Robertson HA (1992b) Synergistic interactions of D1- and D2-selective dopamine agonists in animal models for Parkinson's disease: Sites of action and implications for the pathogenesis of dyskinesias. *Can J Neurol Sci* 19 (1 Suppl): 147-152

Rodriguez MC, Guridi OJ, Alvarez L, Mewes K, Macias R, Vitek J, DeLong, MR, Obeso JA (1998) The subthalamic nucleus and tremor in Parkinson's disease. *Mov Disord* 13 (Suppl 3): 111-118

Rosenblad C, Kirik D, Björklund (1999) Neurturin enhances the survival of intrastriatal fetal dopaminergic transplants. *Neuroreport* 10: 1783-1787

Rosenblad C, Martinez-Serrano A, Björklund A (1996) Glial cell line-derived neurotrophic factor increases survival, growth and function of intrastriatal fetal nigral dopaminergic grafts. *Neuroscience* 75: 979-985

Sakurada K, Ohshima-Sakurada M, Palmer TD, Gage FH (1999) Nurr1, an orphan nuclear receptor, is a transcriptional activator of endogenous tyrosine hydroxylase in neural progenitor cells derived from the adult brain. *Development* 126: 4017-4026

Salo PT, Tatton WG (1992) Deprenyl reduces the death of motoneurons caused by axotomy. *J Neurosci Res* 31: 394-400

Saltykow S (1905) Verusche uber Gehirn replantation, Zugleich ein Beitrag zur Kenntniss reactiver Vorgange an den zelligen Gehirneelementen. *Arch Psychiatr Nervenkr* 40: 329-388

Saporta S, Borlongan CV, Sanberg PR (1999) Neural transplantation of human teratocarcinoma (hNT) neurons into ischemic rats. A quantitative dose-response analysis of cell survival and behavioural recovery. *Neuroscience* 91: 519-525

Saucedo-Cardenas O, Quintana-Hau JD, Le WD, Smidt MP, Cox JJ, De Mayo F, Burbach JP, Conneely OM (1998) Nurr1 is essential for the induction of the dopaminergic phenotype and the survival of ventral mesencephalic late dopaminergic precursor neurons. *Proc Natl Acad Sci USA* 95: 4013-4018

Sautter J, Meyer M, Spenger C, Seiler RW, Widmer HR (1998a) Effects of combined BDNF and GDNF treatment on cultured dopaminergic midbrain neurons. *Neuroreport* 9: 1093-1096

Sautter J, Tseng JL, Braguglia D, Aebischer P, Spenger C, Seiler RW, Widmer HR, Zurn AD (1998b) Implants of polymer-encapsulated genetically modified cells releasing glial cell line-derived neurotrophic factor improve survival, growth, and function of fetal dopaminergic grafts. *Exp Neurol* 149: 230-236

Schell GR, Strick PL (1984) The origin of thalamic inputs to the arcuate premotor and supplementary motor areas. *J Neurosci* 4: 539-560

Schierle GS, Hansson O, Leist M, Nicotera P, Widner H, Brundin P (1999) Caspase inhibition reduces apoptosis and increases survival of nigral transplants. *Nat Med* 5: 97-100

Schinstine M, Rosenberg MB, Routledge-Ward C, Friedmann T, Gage FH (1992) Effects of choline and quiescence on *Drosophila* choline acetyltransferase expression and acetylcholine production by transduced rat fibroblasts. *J Neurochem* 58: 2019-2029

Schmidt RH, Björklund A, Stenevi, Dunnett SB, Gage FH (1983) Intracerebral grafting of neuronal cell suspensions. III. Activity of intrastriatal nigral suspension implants as assessed by measurements of dopamine synthesis and metabolism. *Acta Neurol Scand Suppl* 522: 19-28

Schmidt WJ (1995) Balance of neurotransmitter activities in the basal ganglia loops. *J Neural Transm* 46 (Suppl): 67-76

Schrag A, Schelosky L, Scholz U, Poewe W (1999) Reduction of Parkinsonian signs in patients with Parkinson's disease by dopaminergic versus anticholinergic single-dose challenges. *Mov Disord* 1999 14: 252-255

Scott WK, Yamaoka LH, Stajich JM, Scott BL, Vance JM, Roses AD, Pericak-Vance MA, Watts RL, Nance M, Hubble J, Koller W, Stern MB, Colcher A, Allen FH Jr, Hiner BC, Jankovic J, Ondo W, Laing NG, Mastaglia F, Goetz C, Pappert E, Small GW, Masterman D, Haines JL, Davies TL (1999) The alpha-synuclein gene is not a major risk factor in familial Parkinson disease. *Neurogenetics* 2: 191-192

Segovia J, Castro R, Notario V, Gale K (1991) Transplants of fetal substantia regulate glutamic acid decarboxylase gene expression in host striatal neurons. *Brain Res Mol Brain Res* 10: 359-362

Segovia J, Vergara P, Brenner M (1998) Differentiation-dependent expression of transgenes in engineered astrocyte cell lines. *Neurosci Lett* 242: 172-176

Seidler A, Hellenbrand W, Robra BP, Vieregge P, Nischan P, Joerg J, Oertel WH, Ulm G, Schneider E (1996) Possible environmental, occupational, and other etiologic factors for Parkinson's disease: A case-control study in Germany. *Neurology* 46: 1275-1284

Semba K, Fibiger HC (1992) Afferent connections of the laterodorsal and the pedunculopontine tegmental nuclei in the rat: A retro- and antero-grade transport and immunohistochemical study. *J Comp Neurol* 323: 387-410

Semchuk KM, Love EJ (1995) Effects of agricultural work and other proxy-derived case-control data on Parkinson's disease risk estimates. *Am J Epidemiol* 41:747-754

Seniuk NA, Henderson JT, Tatton WG, Roder JC (1994) Increased CNTF gene expression in process-bearing astrocytes following injury is augmented by R(-)-deprenyl. *J Neurosci Res* 37: 278-286

Shimura H, Hattori N, Kubo S, Yoshikawa M, Kitada T, Matsumine H, Asakawa S, Minoshima S, Yamamura Y, Shimizu N, Mizuno Y (1999) Immunohistochemical and subcellular localization of Parkin protein: absence of protein in autosomal recessive juvenile parkinsonism patients. *Ann Neurol* 45: 668-672

Siegfried J, Lippitz, B (1994) Bilateral chronic electrostimulation of ventroposterolateral pallidum: a new therapeutic approach for alleviating all Parkinsonian symptoms. *Neurosurgery* 35: 1126-1130

Simon H, Taghzouti K, Gozlan H, Studler JM, Louilot A, Herve D, Glowinski J, Tassin JP, Le Moal M (1988) Lesion of dopaminergic terminals in the amygdala produces enhanced locomotor response to D-amphetamine and opposite changes in dopaminergic activity in prefrontal cortex and nucleus accumbens. *Brain Res* 447: 335-340

Simonds GR, Freed WJ (1990) Effects of intraventricular substantia nigra allografts as a function of donor age. *Brain Res* 530: 12-19

Sinclair SR, Svendsen CN, Torres EM, Martin D, Fawcett JW, Dunnett SB (1996) GDNF enhances dopaminergic cell survival and fibre outgrowth in embryonic nigral grafts. *Neuroreport* 7: 2547-2552

Sirinathsinghji DJ, Dunnett SB (1991) Increased proenkephalin mRNA levels in the rat neostriatum following lesion of the ipsilateral nigrostriatal dopamine pathway with 1-methyl-4-phenylpyridinium ion (MPP+): Reversal by embryonic nigral dopamine grafts. *Brain Res Mol Brain Res* 9: 263-269

Sladek JR Jr, Collier TJ, Haber SN, Deutch AY, Elsworth JD, Roth RH, Redmond DE Jr (1987) Reversal of parkinsonism by fetal nerve cell transplants in primate brain. *Ann NY Acad Sci* 495: 641-657

Sladek JR Jr, Collier TJ, Haber SN, Roth RH, Redmond DE Jr (1986) Survival and growth of fetal catecholamine neurons transplanted into primate brain. *Brain Res Bull* 17: 809-818

Sladek JR Jr, Redmond DE Jr, Collier TJ, Blount JP, Elsworth JD, Taylor JR, Roth RH (1988) Fetal dopamine grafts: Extended reversal of methyphenyltetrahydropyridine-induced parkinsonism in monkeys. *Prog Brain Res* 78: 497-506

Smith TS, Parker Jr WD, Bennett Jr JP (1994) L-DOPA increases nigral production of hydroxyl radicals *in vivo*: Potential L-DOPA toxicity? *Neuroreport* 5: 1009-1011

Smith Y, Parent A (1988) Neurons of the subthalamic nucleus in primates display glutamate but not GABA immunoreactivity. *Brain Res* 453: 353-356

Soukoup VM, Ingram F, Schiess MC, Bonnen JC, Nauta HJW, Calverley JR (1997) Cognitive sequelae of unilateral posteroventral pallidotomy. *Arch Neurol* 54: 947-950

Spann BM, Grofova I (1991) Nigropedunculopontine projection in the rat: An anterograde tracing study with phaseolus vulgaris-leucoagglutinin (PHA-L). *J Comp Neurol* 311: 375-388

Spencer DD, Robbins RJ, Naftolin F, Marek KL, Vollmer T, Leranth C, Roth RH, Price LH, Gjedde A, Bunney BS, Sass KJ, Elsworth JD, Kier EL, Makuch R, Hoffer PB, Redmond Jr, DE (1992) Unilateral transplantation of human ventral mesencephalic tissue into the caudate nucleus of patients with Parkinson's disease. *N Engl J Med* 327: 1541-1548

Steinbusch HW, Vermeulen RJ, Tonnaer JA (1990) Basic fibroblast growth factor enhances survival and sprouting of fetal dopaminergic cells implanted in the denervated rat caudate-putamen: Preliminary observations. *Prog Brain Res* 82: 81-86

Sterio D, Beric A, Dogali M, Fazzini E, Alfaro G, Devinsky O (1994) Neurophysiological properties of pallidal neurons in Parkinson's disease. *Ann Neurol* 35: 586-591

Studer L, Tabar V, McKay RD (1998) Transplantation of expanded mesencephalic precursors leads to recovery in parkinsonian rats. *Nat Neurosci* 1: 290-295

Stull ND, Iacovitti L (1996) Acidic fibroblast growth factor and catecholamines synergistically up-regulate tyrosine hydroxylase activity in developing and damaged dopamine neurons in culture. *J Neurochem* 67: 1519-1524

Sullivan AM, Pohl J, Blunt SB (1998) Growth/differentiation factor 5 and glial cell line-derived neurotrophic factor enhance survival and function of dopaminergic grafts in a rat model of Parkinson's disease. *Eur J Neurosci* 10: 3681-3688

Sutton JP, Couldwell W, Lew MF, Mallory L, Grafton S, DeGiorgio C, Welsh M, Apuzzo MLJ, Ahmadi J, Waters CH (1995) Ventroposterior medial pallidotomy in patients with advanced Parkinson's disease. *Neurosurgery* 36: 1112-1116

Svendsen CN, Caldwell MA, Shen J, ter Borg MG, Rosser AE, Tyers P, Karmiol S, Dunnett SB (1997) Long-term survival of human central nervous system progenitor cells transplanted into a rat model of Parkinson's disease. *Exp Neurol* 148: 135-146

Svendsen CN, Clarke DJ, Rosser AE, Dunnett SB (1996) Survival and differentiation of rat and human epidermal growth factor-responsive precursor cells following grafting into the lesioned adult central nervous system. *Exp Neurol* 137: 376-388

Sziráki I, Kardos V, Patthy M, Pátfalusi, Gaál J, Solti M, Kollár, Singer J (1994) Amphetamine-metabolites of deprenyl involved in protection against neurotoxicity induced by MPTP and 2'-methyl-MPTP. *J Neural Transm* 41 (Suppl): 207-219

Tai IT, Sun AM (1993) Microencapsulation of recombinant cells: A new delivery system for gene therapy. *FASEB J* 7: 1061-1069

Takayama H, Ray J, Raymon HK, Baird A, Hogg J, Fisher LJ, Gage FH (1995) Basic fibroblast growth factor increases dopaminergic graft survival and function in a rat model of Parkinson's disease. *Nat Med* 1: 53-58

Tasker RR, Lang AE, Lozano AM (1997) Pallidal and thalamic surgery for Parkinson's disease. *Exp Neurol* 144: 35-40

Tasker RR, Siqueira J, Hawrylyshyn P, Organ P (1983) What happened to Vim thalamotomy for Parkinson's disease? *Appl Neurophysiol* 46: 68-83

Tatton WG (1993) Selegiline can mediate neuronal rescue rather than neuronal protection. *Mov Disord* 8 (Suppl 1): S20-S30

Tatton WG, Chalmers-Redman RME (1996) Modulation of gene expression rather than monoamine oxidase inhibition: (-)-deprenyl-related compounds in controlling neurodegeneration. *Neurology* 47 (Suppl 3): S171-S183

Terao T, Yanagihara N, Abe K, Izumi F (1992) Lithium chloride stimulates catecholamine synthesis and secretion in cultured bovine adrenal meduallary cells. *Biol Psychiatry* 31: 1038-1049

Tetrud JW & Langston JW (1989) The effect of deprenyl (selegiline) on the natural history of Parkinson's disease. Science 245: 519-524

Thajeb P, Ling ZD, Potter ED, Carvey PM (1997) The effects of storage conditions and trophic supplementation on the survival of fetal mesencephalic cells. *Cell Transplant* 6: 297-307

Thompson WG (1890) Successful brain grafting. *NY Med J* 51: 701

Tornatore C, Baker-Cairns B, Yadid G, Hamilton R, Meyers K, Atwood W, Cummins A, Tanner V, Major E (1996) Expression of tyrosine hydroxylase in an immortalized human fetal astrocyte cell line: *In vitro* characterization and engraftment into the rodent striatum. *Cell Transplant* 5: 145-163

Trejo F, Vergara P, Brenner M, Segovia J (1999) Gene therapy in a rodent model of Parkinson's disease using differentiated C6 cells expressing a GFAP-tyrosine hydroxylase transgene. *Life Sci* 65: 483-491

Tresco PA, Winn SR, Tan S, Jaeger CB, Greene LA, Aebischer P (1992) Polymer-encapsulated PC12 cells: Long-term survival and associated reduction in lesion-induced rotational behavior. *Cell Transplant* 1: 255-264

Trojanowski JQ, Kleppner SR, Hartley RS, Miyazono M, Fraser NW, Kesari S, Lee VM-Y (1997) Transfectable and transplantable postmitotic human neurons: A potential "platform" for gene therapy of nervous system diseases. *Exp Neurol* 144: 92-97

Trojanowski JQ, Mantione JR, Lee JH, Seid DP, You T, Inge LJ, Lee VM-Y (1993) Neurons derived from a human teratocarcinoma cell line establish molecular and structural polarity following transplantation into the rodent brain. *Exp Neurol* 122: 283-294

Tronnier VM, Fogel W, Kronenbuerger M, Steinvorth S (1997) Pallidal stimulation: An alternative to pallidotomy? *J Neurosurg* 87: 700-705

Turner RS, Suzuki N, Chyung AS, Younkin, Lee VM-Y (1996) Amyloids β40 and β42 are generated intracellularly in cultured human neurons and their secretion increases with maturation. *J Biol Chem* 271: 8966-8970

Verhagen Metman L, Blanchet PJ, van den Munckhof P, Del Dotto P, Natte R, Chase TN (1998a) A trial of dextromethorphan in parkinsonian patients with motor response complications. *Mov Disord* 13: 414-417

Verhagen Metman L, Del Dotto P, Blanchet PJ, van den Munckhof P, Chase TN (1998b) Blockade of glutamatergic transmission as treatment for dyskinesias and motor fluctuations in Parkinson's disease. *Amino Acids* 14: 75-82

Vertes RP (1991) A PHA-L analysis of ascending projections of the dorsal raphe nucleus in the rat. *J Comp Neurol* 313: 643-665

Wagner J, Akerud P, Castro DS, Holm PC, Canals JM, Snyder EY, Perlmann T, Arenas E (1999) Induction of a midbrain dopaminergic phenotype in Nurr1-overexpressing neural stem cells by type 1 astrocytes. *Nat Biotechnol* 17: 653-659

Wang MZ, Jin P, Bumcrot DA, Marigo V, McMahon AP, Wang EA, Woolf T, Pang K (1995) Induction of dopaminergic neuron phenotype in the midbrain by Sonic hedgehog protein. *Nat Med* 1: 1184-1188

Wang WW, Khajavi M, Patel BJ, Beach J, Jankovic J, Ashizawa T (1998) The G209A mutation in the alpha-synuclein gene is not detected in familial cases of Parkinson disease in non-Greek and/or Italian populations. *Arch Neurol* 55: 1521-1523

Wang Y, Tien LT, Lapchak PA, Hoffer BJ (1996) GDNF triggers fiber outgrowth of fetal ventral mesencephalic grafts from nigra to striatum in 6-OHDA-lesioned rats. *Cell Tissue Res* 286: 223-233

Wasielewski PG, Burns JM, Koller WC (1998) Pharmacologic treatment of tremor. *Mov Disord* 13 (Suppl 3): 90-100

Waters C, Itabashi HH, Apuzzo MLJ, Weiner LP (1990) Adrenal to caudate transplantation – a postmortem study. *Mov Disord* 5: 248-250

Weiss S, Reynolds BA, Vescovi AL, Moshead C, Craig CG, van der Kooy D (1996) Is there a neural stem cell in the mammalian forebrain?*Trends Neurosci* 19: 387-393

Wenning GK, Odin P, Morrish P, Rehncrona S, Widner H, Brundin P, Rothwell JC, Brown R, Gustavii B, Hagell P, Jahanshahi M, Sawle G, Björklund A, Brooks DJ, Marsden CD, Quinn NP, Lindvall O (1997) Short- and long-term survival and function of unilateral intrastriatal dopaminergic grafts in Parkinson's disease. *Ann Neurol* 42: 95-107

Wertkin AM, Turner RS, Pleasure SJ, Golde TE, Younkin SG, Trojanowski JQ, Lee VM-Y (1993) Human neurons derived from a teratocarcinoma cell line express solely the 695-amino acid amyloid precursor protein and produce intracellular β-amyloid or A4 peptides. *Proc Natl Acad Sci USA* 90: 9513-9517

Wichmann T, Bergman H, DeLong MR (1994) The primate subthalamic nucleus. III. Changes in motor behaviour and neuronal activity in the internal pallidum induced by subthalamic inactivation in the MPTP model of Parkinsonism. *J Neurophysiol* 72: 521-530

Widner H, Tetrud J, Rehncrona S, Snow B, Brundin P, Gustavii B, Björklund A, Lindvall O, Langston JW (1992) Bilateral fetal mesencephalic grafting in two patients with Parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *N Engl J Med* 327: 1556-1563

Wilby MJ, Sinclair SR, Muir EM, Zietlow R, Adcock AH, Horellou P, Rogers JH, Dunnett SB, Fawcett JW (1999) A glial cell line-derived neurotrophic factor-secreting clone of the Schwann cell line SCTM41 enhances survival and fiber outgrowth from embryonic nigral neurons grafted to the striatum and lesioned substantia nigra. *J Neurosci* 19: 2301-2312

Williams SM, Goldman-Rakic PS (1998) Widespread origin of the primate mesofrontal dopamine system. *Cereb Cortex* 8: 321-45

Winkler C, Bentlage C, Nikkhah G, Samii M, Björklund A (1999) Intranigral transplants of GABA-rich striatal tissue induce behavioral recovery in the rat Parkinson model and promote the effects obtained by intrastriatal dopaminergic transplants. *Exp Neurol* 155: 165-186

Wolff JA, Fisher LJ, Xu L, Jinnah HA, Langlais PJ, Iuvone PM, O'Malley KL, Rosenberg MB, Shimohama S, Friedman T, Gage FH (1989) Grafting fibroblasts genetically modified to produce L-dopa in a rat model of Parkinson disease. *Proc Natl Acad Sci USA* 86: 9011-9014

Wu CY, De Zhou MD, Bao XF, Zhang QL, Sun W, Li FZ, Zhao JJ (1994) The combined method of transplantation of foetal substantia nigra and stereotactic thalamotomy for Parkinson's disease. *Br J Neurosurg* 8: 709-716

Wu R-M, Murphy DL, Chiueh CC (1996) Suppression of hydroxyl radical formation and protection of nigral neurons by l-deprenyl (Selegiline). *Ann NY Acad Sci* 786: 379-390

Yasui Y, Tsumori T, Ando A, Domoto T (1996) A nigro-rubro-bulbar pathway to the parvicellular reticular formation in the rat. *Neuroreport* 7: 1157-1160

Yokoyama T, Sugiyama K, Nishizawa S, Yokota N, Ohta S, Uemura K (1999) Subthalamic nucleus stimulation for gait disturbance in Parkinson's disease. *Neurosurgery* 45: 41-47

Youdim MBH, Riederer P (1997) Understanding Parkinson's disease. *Sci. Amer* 276: 52-59

Younkin DP, Tang CM, Hardy M, Reddy UR, Shi QY, Pleasure SJ, Lee VM-Y, Pleasure D (1993) Inducible expression of neuronal glutamate receptor channels in the NT2 human cell line. *Proc Natl Acad Sci USA* 90: 2174-2178

Yurek DM (1998) Glial cell line-derived neurotrophic factor improves survival of dopaminergic neurons in transplants of fetal ventral mesencephalic tissue. *Exp Neurol* 153: 195-202

Yurek DM, Lu W, Hipkens S, Wiegand SJ (1996) BDNF enhances the functional reinnervation of the striatum by grafted fetal dopamine neurons. *Exp Neurol* 137: 105-118

Zawada WM, Zastrow DJ, Clarkson ED, Adams FS, Bell KP, Freed CR (1998) Growth factors improve immediate survival of embryonic dopamine neurons after transplantation into rats. *Brain Res* 786: 96-103

Zeng BY, Jenner P, Marsden CD (1996) Partial reversal of increased preproenkephalin messenger ribonucleic acid (mRNA) and decreased preprotachykinin mRNA by foetal dopamine cells in unilateral 6-hydroxydopamine-lesioned rat striatum parallels functional recovery. *Mov Disord* 11: 43-52

Zigova T, Pencea V, Betarbet R, Wiegand SJ, Alexander C, Bakay RA, Luskin MB (1998) Neuronal progenitor cells of the neonatal subventricular zone differentiate and disperse following transplantation into the adult rat striatum. *Cell Transplant* 7: 137-156

Zigova T, Willing AE, Tedesco EM, Borlongan CV, Saporta S, Snable GL, Sanberg PR (1999) Lithium chloride induces the expression of tyrosine hydroxylase in hNT neurons. *Exp Neurol* 157: 251-258