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PRINCIPAL INVESTIGATOR: Ole Isacson, M.D.

CONTRACTING ORGANIZATION: The McLean Hospital Corporation
Belmont, Massachusetts 02178-9106

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Ole Isacson, M.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The McLean Hospital Corporation
Belmont, Massachusetts 02178-9106

e-mail:
isacson@helix.mgh.harvard.edu

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13. ABSTRACT *(Maximum 200 Words)*

This work develops a methodology to assess the acute toxicity induced by the MPTP treatment in a neurotoxic model of Parkinson's disease. Secondly, the progressive loss of dopamine axons can be diagnostically measured by positron emission tomography (PET) and using specific ligands such as CFT. Third, we are addressing whether xenogeneic dopamine neurons can replace the neurons lost by the neurotoxic process. We will also compare neurotransplantation with pallidotomy. The current analysis is dependent upon behavioral, PET, MRI/MRS and finally, post-mortem methodology to determine the questions and objectives outlined in this plan. In this period, we have found through combined PET and MRS studies how neurochemical changes are linked in the primate model for Parkinson's disease. We have obtained specific changes that provide us with predictive mathematical models for the progressive degeneration in Parkinson's disease. These results provide a useful model and novel diagnostic tools for neurotoxically induced Parkinson's disease.

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FOREWORD

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INTRODUCTION:

This work develops new functional diagnostics and treatments for Parkinson's disease (PD) from pre-clinical experiments in primate models of neurotoxically induced PD. Given that (1) dopamine (DA) neurons die and a stable PD-like behavioral syndrome appears in primates after chronic administration of a neurotoxin: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), (2) loss of dopaminergic axon can be diagnostically detected by positron emission tomography (PET) and ligands to label striatal DA reuptake sites, (3) neural transplantation may replace neurotoxically eliminated neurons and reverse PD-like symptoms and drug induced side effects, we will now determine how implanted fetal porcine neural DA and control non-DA cells can repair neural systems and reverse behavioral deficits. Pallidotomy is tested as a parallel therapeutic method. We will measure DA receptors and cerebral oxidative glucose metabolism by PET and neuroanatomy, hemodynamics, levels and profiles of brain tissue neurochemicals by MRI/MRS in rodent and primate animal models. The data-sets from PET and MRI/MRS are correlated with behavioral and post-mortem studies. This project develops 1) objective *in vivo* measurements of brain damage associated with neurotoxins and 2) therapies for neurotoxically induced PD.

BODY:

We describe below the research accomplishments associated with the approved Statement of Work, which is copied here in bold. The publications and figures referenced are attached in the Appendix.

STATEMENT OF WORK

WE WILL DETERMINE AND DEVELOP NOVEL DIAGNOSITC CRITERIA FOR ACUTE NEUROTOXICITY AND LONG-TERM DEGENERATION OF THE DOPAMINE SYSTEM (OBJECTIVE 1)

Starting in year 1, and continuing through year 3, we determine in MPTP induced primate parkinsonism, the consequences of acute neurotoxicity (ANT). The following questions are answered in this sequence:

Step 1.1. Are there changes in dopamine reuptake sites or dopamine receptors in ANT?

Our results indicate that there is a rapid loss of dopamine reuptake sites and corresponding upregulation of dopamine receptors in ANT. (Fig. 2a, appendix).

Step 1.2. Is there any sign of oxidative stress in ANT?

Our results indicate that there are dramatic signs of oxidative stress in ANT that (see below) continues after chronic loss of dopamine neurons. (Brownell et al 1998, 1999, appendix).

Step 1.3. Are there changes in tissue neurochemical profiles in ANT?

Our results indicate that there are initial progressive changes in the neurochemical profile such that while there are increases in choline and decreases in NAA, there are parallel increases in lactate and macromolecules paralleling Parkinson's disease. (Brownell et al 1998 and Fig. 1-2, appendix).

Step 1.4. Are there hemodynamic changes observable in ANT?

This work is in progress.

Step 1.5. Is there any change in behavioral locomotor activity in ANT?

Our results indicate that there is a rapid loss of locomotor activity which parallels the neurotoxic syndrome. However, this change does not become overt Parkinsonism until a chronic stage of at least 70-80% loss of dopamine. (Brownell et al 1998, 1999, appendix).

The specific biological questions during longer-term degeneration are studied in years 2-4:
Step 1.6. Does the initial DA loss trigger metabolic and/or neurochemical changes over time in non-DA systems?

The initial dopamine loss triggers long-term changes in non-dopamine systems. We have found that after neurotoxic loss, there are MRI/MRS changes that are detectable up to two and a half years after the end of the neurotoxin treatment. Similarly, the initial loss of dopamine induces a cascade of degenerations that persist for and terminate years after the initial toxic exposure. (Brownell et al 1998, 1999, appendix).

Step 1.7 As an endpoint of stable Parkinsonism longitudinal studies will be correlated with clinically relevant behavior in a slowly progressing primate Parkinson disease model.

These studies are ongoing and indicate that Parkinson's disease is mirrored very closely by MPTP toxin treatment.

The PET studies using ^{11}CFT show the binding to dopamine reuptake sites and ^{11}C-raclopride to dopamine D_2 receptors. Oxidative stress was observed by PET studies

of oxidative metabolism (oxygen extraction fraction, oxygen metabolism and glucose metabolism) as well as MRS studies of lactate/lipid peaks. MRS studies simultaneously show a number of tissue neurochemicals: choline, creatine, N-acetylaspartate, myo-inositol. Functional MR imaging will provide maps of hemodynamic indices over the entire brain. Locomotor activity is measured in parallel.

IN EXPERIMENTAL PD MODELS, WE WILL DETERMINE THE MECHANISMS BEHIND EFFECTS OF THERAPEUTIC INTERVENTIONS WITH FETAL NEURONS OR PALLIDOTOMY (OBJECTIVE 2)

Therapeutic interventions will be investigated in combination with PET and MRI/MRS and locomotor activity studies. Initiated in year 1, but continuing through year 4 we will answer the following biological questions in a primate PD model:

Step 2.1. Is there change in dopamine reuptake sites or dopamine receptors after transplantation with DA or non-DA neurons?

Summary of transplantation data provided in Fig. 3. We are in progress on PET scanning for grafted animals.

Step 2.2. Has oxidative stress recovered after transplantation with DA or non-DA neurons?

The initial studies on xenogeneic transplantation in this Parkinson's model are in progress (n=1), but preliminary data suggest a normalization.

Step 2.3. Are there changes in tissue neurochemical profiles after transplantation with DA or non-DA neurons?

Tissue neurochemical profiles when there are surviving grafts appear to be normalized by the transplantation.

Step 2.4. Is there vascular arborization after transplantation with DA or non-DA neurons?

The initial studies on xenogeneic transplantation in this Parkinson's model are in progress.

Step 2.5.Is there change in locomotor activity after transplantation with DA or non-DA neurons?

The initial studies on xenogeneic transplantation in this Parkinson's model are in progress.

Step 2.6. Does pallidotomy effect on regional blood flow, oxygen extraction fraction, oxygen or glucose metabolism?

These studies are in progress.

Step 2.7. Does pallidotomy have any effect on dopamine reuptake sites or dopamine receptors?

These studies are in progress.

Step 2.8. Does pallidotomy have any effect on neurochemicals?

These studies are in progress.

Step 2.9. Does pallidotomy effect on behavioral locomotor activity?

These studies are in progress.

Step 2.10. The endpoint correlation of parameters derived of imaging studies with behavioral studies and post-mortem histology.

These studies are in progress. Preliminary data indicate that the imaging studies are highly predictive of the postmortem analysis of remaining or degenerated dopamine fibers.

KEY RESEARCH ACCOMPLISHMENTS:

- Neurotoxin treatment with oxidative stress with complex I inhibitors (toxin: MPTP) creates a syndrome identical to Parkinson's disease
- The acute neurotoxic treatment with MPTP creates an immediate loss of dopamine terminals and a compensatory up-regulation of dopamine receptors
- Magnetic resonance spectroscopy (MRS) indicates a 23-fold increase in lactate and macromolecules that persist for up to 10 months after neurotoxin administration
- The MRS lactate and macromolecule values return to normal by two years after the final MPTP toxin exposure.
- There are persistent increases in striatal choline (gliosis and inflammatory response) and decreases in NAA (loss of dopaminergic and neuronal elements for chronic periods extend beyond two years).
- The progressive loss of dopamine terminals following neurotoxin exposure follows an exponential curve and a mathematical model similar to cell survival theory.

- The MPTP primate model has MRI and MRS spectra similar to Parkinson's patients. The predictive value of the equations for this degeneration phenomena provide an opportunity for protective treatments.
- These neurotoxin models with the oxidative damage simulate all known aspects of idiopathic Parkinson's disease.

REPORTABLE OUTCOMES:

Manuscripts:

1. Isacson, O., Deacon, T. and Schumacher, J. (1998) Immunobiology and Neuroscience of Xenotransplantation in Neurological Disease. In: CNS Regeneration: Basic Science and Clinical Advances, M.H. Tuszynski and J.H. Kordower, eds., Academic Press, San Diego, pp. 365-387.
2. Brownell, A.-L., Jenkins, B.G., Elmaleh, D.R., Deacon, T.W., Spealman, R.D., Isacson, O. (1998) Combined PET/MRS studies of the brain reveal dynamic and long-term physiological changes in a Parkinson's disease primate model. Nature Med. 4, 1308-1312.
3. Costantini, L.C. and Isacson, O.(1999) Dopamine neuron grafts: development and molecular biology. In: Dopamine Neuron Development, U. di Porzio, R. Pernas-Alonso and C. Perone-Capano, eds., R.G. Landes Company, Georgetown, in press.
4. Isacson, O. and Sladek, J. (1999) Cellular and Molecular Treatments of Neurological Diseases. Exp. Neurol. 159, 1-3.
5. Brownell, A-L, Jenkins, B. and Isacson, O. (1999) Dopamine Imaging Markers and Predictive Mathematical Models for Progressive Degeneration in Parkinson's Disease. Biomedicine & Pharmacotherapy 53, 131-140.
6. Fink, J.S., Schumacher, J.M., Ellias, S.L., Palmer, E.P., Saint-Hilaire, M., Shannon, K., Penn, R., Starr, P., van Horne, C., Kott, H.S., Dempsey, P.K., Fischman, A.J., Raineri, R., Manhart, C., Dinsmore, J., Isacson, O. (1999) Porcine xenografts in Parkinson's disease and Huntington's disease patients: tentative outcomes. Cell Transplant., in press.

Abstracts:

1. A.L. Brownell, B.G. Jenkins, D.R. Elmaleh, T.W. Deacon, O. Isacson, Long-Term In Vivo PET/MRS Neurodegeneration Studies of a Primate Parkinson's Disease Model, Soc. Neurosci. 1998.
2. O. Isacson, Transplantation Approaches in Parkinson's Disease, 5th International Congress of Parkinson's Disease and Movement Disorders, New York City, New York October 10-14,1998.
3. O. Isacson, Cell transplantation as a therapy for Parkinson's Disease, The Physiological Society, Cardiff, Wales, Dec. 18, 1998.
4. O. Isacson, Primary Neuronal Cell Transplantation for Parkinson's Disease, The Cell Transplant Society, Montreux, Switzerland, Mar. 21-24, 1999.

5. O. Isacson, Neural Xenotransplantation for Neurodegenerative Disease, Keystone Symposia on Molecular and Cellular Biology, Lake Tahoe, NV, Mar. 21-26, 1999.
6. T.W. Deacon, W. Fodor, S. Rollins, S. Squinto, L.C. Costantini, L. Matis, L. Bell and O. Isacson. Xenotransplantation of transgenic fetal pig dopamine neurons. American Society for Neural Transplantation, 1999.
7. Isacson O, Deacon TW, Costantini LC & Brownell AL. Animal models of PD: novel neuroprotection and cell implantation paradigms. Intl. Transplant. Soc., Vancouver, BC, Aug. 26-28, 1999.
8. O. Isacson. Dopamine neuron transplantation: pharmacological and behavioral aspects. Behavioral Pharmacology Meeting, Boston, MA, Sept. 1-4, 1999.
9. O. Isacson. Neural Transplantation in Neurodegenerative Diseases. Year of the Brain Intl. Symp., Vienna, Oct. 1-3, 1999.
10. A.-L. Brownell, T. van Nguyen, Y-C. J. Chen, F. Cavagna, B.R. Bosen, O. Isacson, B.Q. Jenkins. PET and phMRI sutides of dopamine receptor modulation in PD models. Soc. Neurosci. 1999.

Presentations:

1998 New York, NY, 5th Intl. Congress of Parkinson's Disease and Movement Disorders. "Gene Therapy for Parkinsons' Disease", (plenary lecture)
1998 Tokyo, Japan,The Molecular Medicine Revolution Conference, "Neural cell transplants to physiologically repair circuitry in neurodegenerative diesease" (plenary lecture)
1998 Cardiff, Wales, The Physiological Society, "Cell transplantation as a therapy for Parkinson's disease" (lecture)
1999 Cornell Medical School/New York Hospital "Developing nerve cells against neurodegeneration" (grand rounds & lecture)
1999 Montreux, Switzerland, The Cell Transplant Society, "Primary neuronal cell transplantation for Parkinson's disease (lecture)
1999 Keystone Symposia, "Neural xenotransplantation for neurodegenerative disease" (lecture)
1999 Dalhousie University, Halifax, Clinical Neuroscience (rounds) and Dept. of Anatomy and Neurobiology (lecture)
1999 University of Pittsburgh Medical Center, Dept. of Pathology (lecture)
1999 University of Rochester, Experimental Therapeutics Workshop (lecture) and Neurology Grand Rounds
1999 Vancouver, BC, XIIIth Intl. Congress on Parkinson's Disease (lecture)
1999 Odense, Denmark, 7th Intl. Neural Transplantation Meeting (lecture)
1999 Boston, European Behavioral Pharmacology Society and Behavioral Pharmacology Society Conference (lecture)
1999 Austrian Parkinson Society, Vienna (lecture)
1999 Bonn, Intl. Neuroscience Symposium "Molecular Basis of CNS Disorders" (lecture)
1999 London, The Novartis Foundation "Neural Transplantation in Neurodegenerative Disease" (Discussant)

CONCLUSIONS:

Our findings using in vivo PET/MRS brain imaging indicate that there are very complex and dynamic changes following toxin exposure that is similar to Parkinson's disease in most respects. The studies provide us with exact mathematical models for which both the degeneration and neuroprotection interventions can be tested. This work is valuable both as an exact theoretical analysis, as well as an indepth study of neurotoxin exposure that creates Parkinson's disease. Many molecules in the environment and potentially administered toxins can simulate the action of these molecules, which in acute or with repeated exposure could increase the risk of Parkinson's disease. The value of this study is also that it allows neuroprotection studies to be initiated and/or neural replacement studies by transplantation to present or repair these toxic changes.

APPENDICES:

Figures 1, 2, 3 and Figure Legends

Publications:

Brownell, A.-L., Livni, E., Galpern, W., and Isacson, O. (1998) In vivo PET imaging in rat of dopamine terminals reveals functional neural transplants. Ann. Neurol., 43, 387-390

Brownell, A-L, Jenkins, B. and Isacson, O. (1999) Dopamine Imaging Markers and Predictive Mathematical Models for Progressive Degeneration in Parkinson's Disease. Biomedicine & Pharmacotherapy 53, 131-140.

Curriculum Vitae: Dr. Ole Isacson

Figure Legends

Figures 1. A follow-up study of comparative distribution of pre-synaptic dopamine transporters with PET using 11C-CFT in a primate Parkinson disease model.
(a) Five representative coronal brain levels (A25, A20, A15, A10 and P5) are presented before (control), 1 and 2 months after acute MPTP treatment. Images show ^{11}C-CFT distribution in the brain 40-45 min after injection of the labeled ligand. ***(b)*** Time-activity curves show time dependent distribution of ^{11}C-CFT in putamen, caudate and cerebellum before and 2 months after MPTP treatment.

Figures 2. A parallel follow-up study (Figures 1) of comparative distribution of post-synaptic dopamine D2 receptors with PET using 11C-raclopride.
(a) Four representative coronal brain levels (A25, A20, A15 and A10) are presented before and 2 months after MPTP treatment. Images show ^{11}C-raclopride distribution in the brain 60-64 min after injection of ^{11}C-raclopride. ***(b)*** Time-activity curves show time dependent distribution of ^{11}C-raclopride in the same brain areas as Figure 1b before and 2 months after MPTP.

Figure 3. CD59 Transgenic Fetal Pig Graft in monkey Mf281.98.
This case was distinguished by continuous cyclosporine delivery via a jugular catheter using an infusion pump. After 9 weeks survival histology demonstrated a large densely compacted graft (see Nissl; far left), that immunostained positive for TH, the pig-specific markers NF70 and CD44, and weakly for the transgene product CD59.

Figure 1a

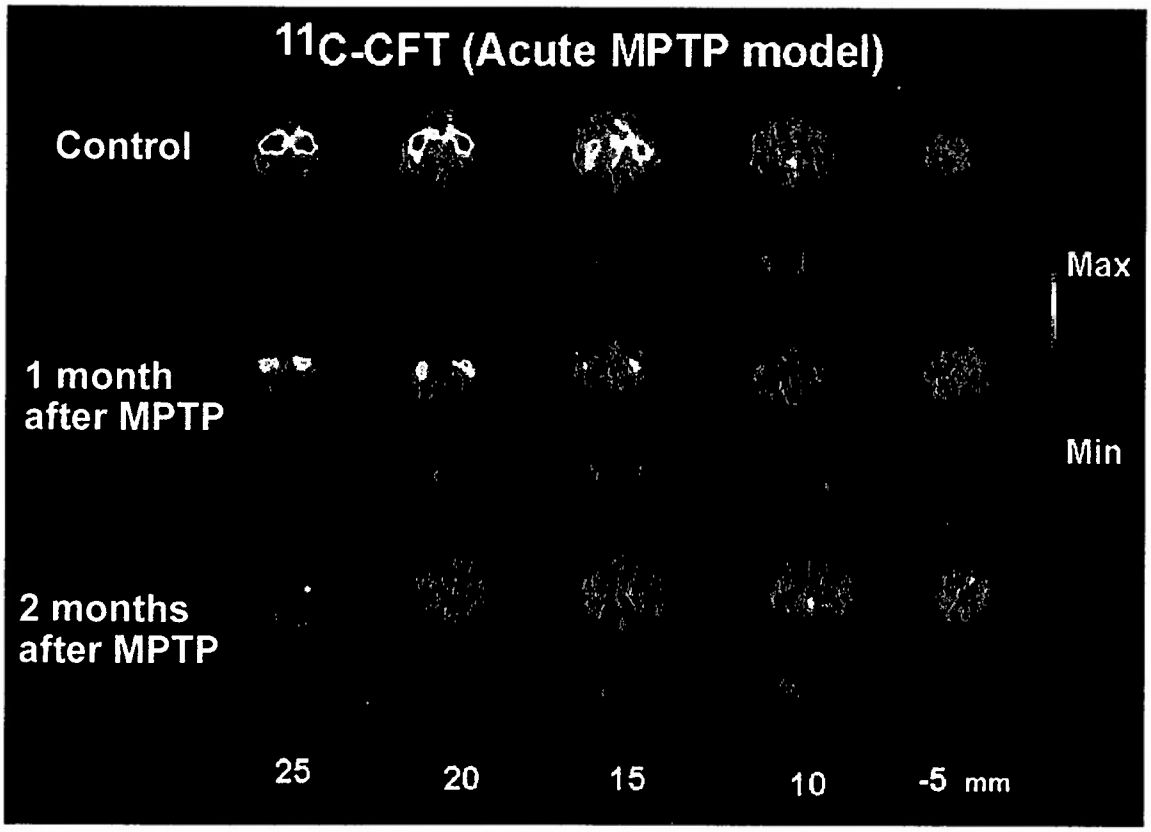

11C-CFT (Acute MPTP model)
Control
1 month after MPTP
2 months after MPTP
Max
Min
25
20
15
10
-5 mm

Figure 1b

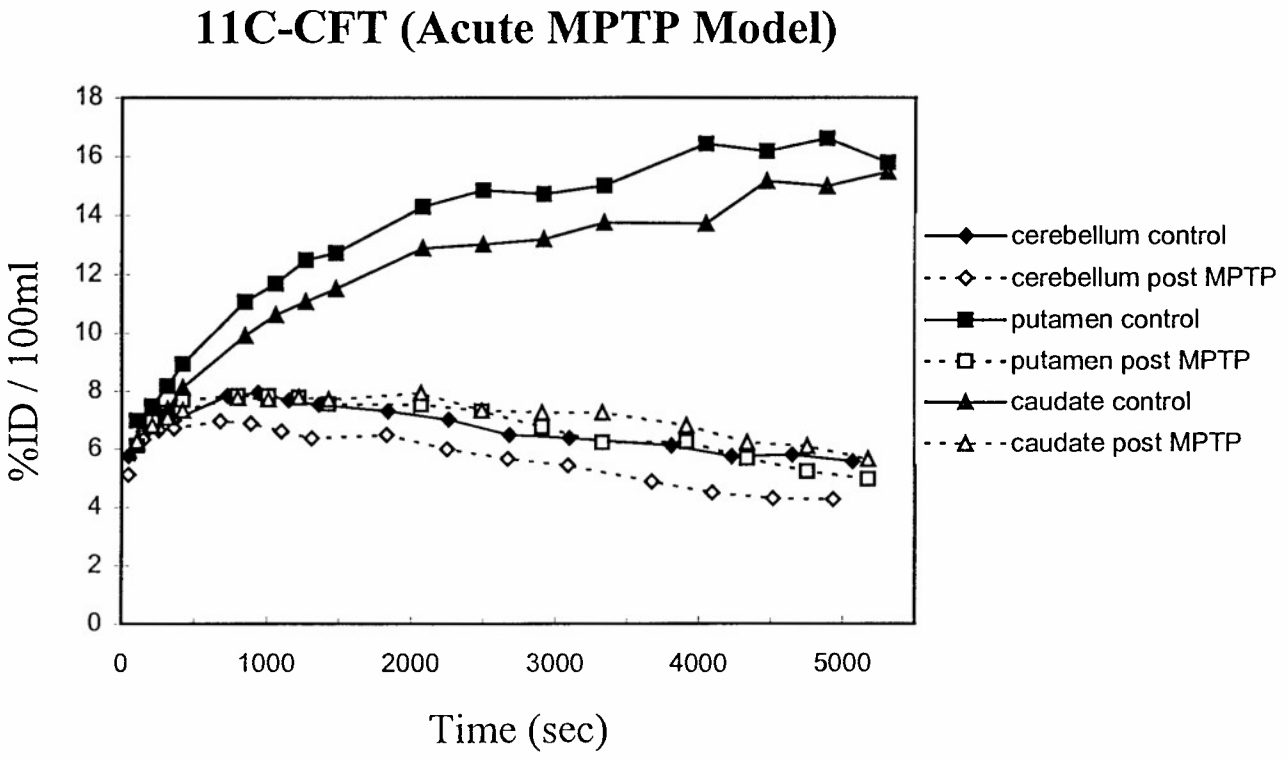

11C-CFT (Acute MPTP Model)
%ID / 100ml
18
16
14
12
10
8
6
4
2
0
0
1000
2000
3000
4000
5000
Time (sec)
cerebellum control
cerebellum post MPTP
putamen control
putamen post MPTP
caudate control
caudate post MPTP

Figure 2a

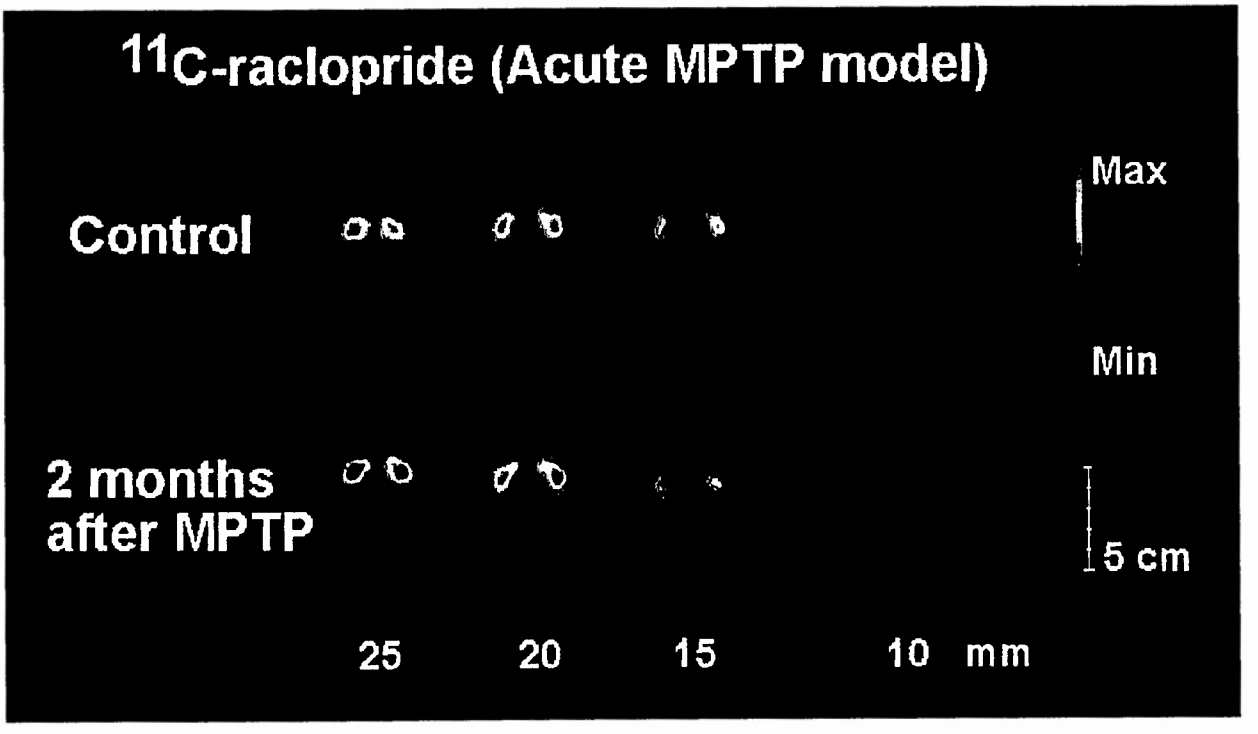

11C-raclopride (Acute MPTP model)
Control
2 months after MPTP
Max
Min
5 cm
25
20
15
10 mm

Figure 2b

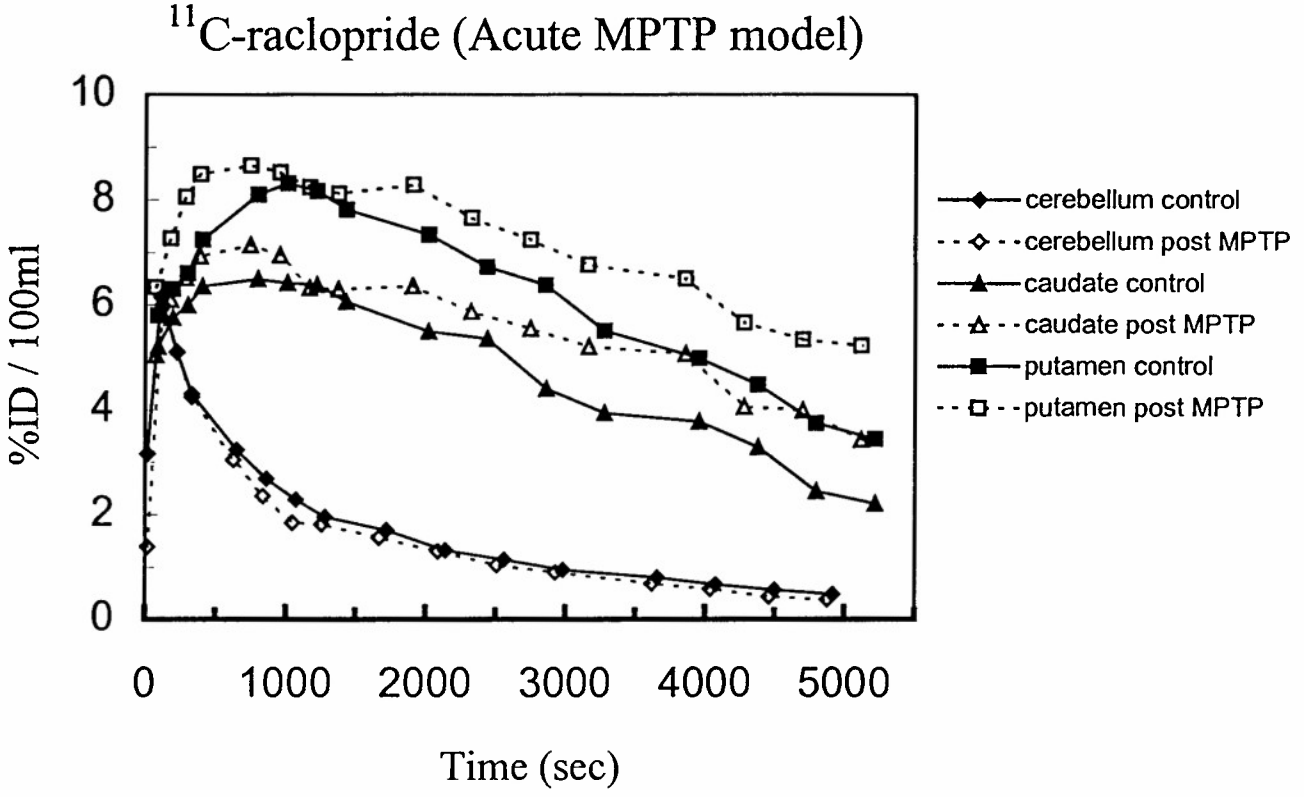
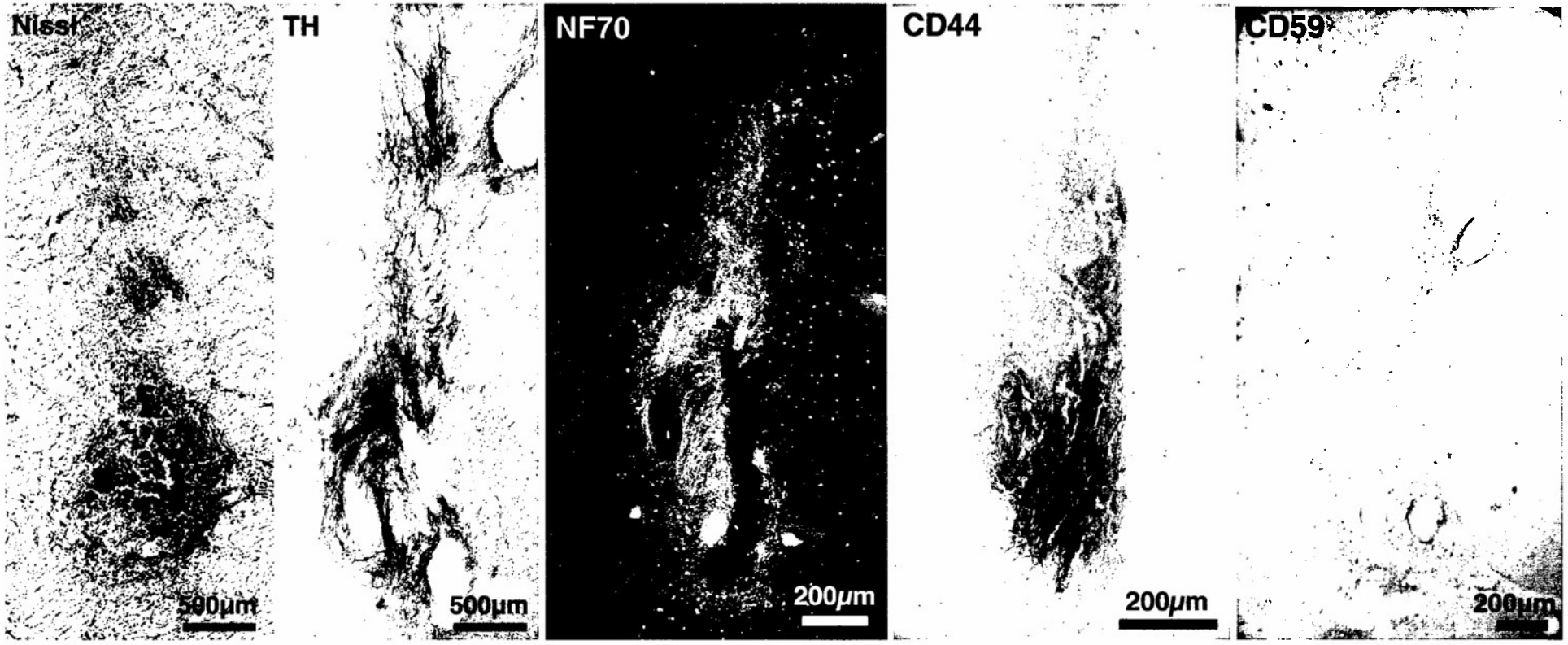

11C-raclopride (Acute MPTP model)
%ID / 100ml
10
8
6
4
2
0
0
1000
2000
3000
4000
5000
Time (sec)
cerebellum control
cerebellum post MPTP
caudate control
caudate post MPTP
putamen control
putamen post MPTP

Figure 3



Combined PET/MRS brain studies show dynamic and long-term physiological changes in a primate model of Parkinson disease

ANNA-LIISA BROWNELL[1,3], BRUCE G. JENKINS[1], DAVID R. ELMALEH[1], TERRENCE W. DEACON[3], ROGER D. SPEALMAN[4] & OLE ISACSON[2,3]

[1]Department of Radiology and [2]Neurology, Massachusetts General Hospital, and Program in Neuroscience Harvard Medical School, Boston, Massachusetts 02114, USA [3]Neuroregeneration Laboratories, McLean Hospital, Belmont, Massachusetts 02178, USA

[4]New England Regional Primate Research Center, Southborough, Massachusetts 01772, USA

Correspondence should be addressed to O.I.

We used brain imaging to study long-term neurodegenerative and bioadaptive neurochemical changes in a primate model of Parkinson disease. We gradually induced a selective loss of nigrostriatal dopamine neurons, similar to that of Parkinson disease, by creating oxidative stress through infusion of the mitochondrial complex 1 inhibitor MPTP for 14 ± 5 months. Repeated evaluations over 3 years by positron emisslon tomography (PET) demonstrated progressive and persistent loss of neuronal dopamine pre-synaptic re-uptake sites; repeated magnetic resonance spectroscopy (MRS) studies indicated a 23-fold increase in lactate and macromolecules in the striatum region of the brain for up to 10 months after the last administration of MPTP. By 2 years after the MPTP Infusions, these MRS striatal lactate and macromolecule values had returned to normal levels. In contrast, there were persistent increases in striatal choline and decreases in N-acetylaspartate. Thus, these combined PET/MRS studies demonstrate patterns of neurochemical changes that are both dynamic and persistent long after selective dopaminergic degeneration.

In neurological diseases like Parkinson disease (PD), examination of the living brain by high resolution positron emission tomography (PET) and magnetic resonance imaging (MR), combined with the appropriate pharmaco-kinetic and physiological analyses, can provide valuable quantitative information of altered brain function[1,2]. Imaging technology depends on the limits of imaging (resolution and sensitivity) as well as biological variables (tissue structure and biochemical processes)(refs. 3–6). In applications involving the human brain, recent progress in obtaining localized magnetic resonance spectra (MRS) and spectroscopic images has made possible new studies of tumors[7,8] and infarcts[9,10], as well as examination of normal brain physiology[11].

The most prominent pathological change in idiopathic Parkinson disease is degeneration of the nigrostriatal-dopaminergic pathway associated with severe cell loss in the substantia nigra[12]. In patients, a chief consequence of the loss of dopamine (DA) neurons is a substantial decrease in the density of dopaminergic synapses and in the concentrations of DA in the striatum[13,14]. The striatal loss of DA results in typical signs, including akinesia, bradykinesia, rigidity and resting tremor. These findings led to experiments aimed at developing animal models of PD using neurotoxins; such as 6-hydroxydopamine[15,16], selective for DA neurons. Some cases of parkinsonism have developed after accidental intravenous self-administration of a meperidine analogue; 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine[17] (MPTP). The affected individuals had symptoms that included severe akinesia, rigidity, flexed posture and a resting tremor. The symptoms were associated with decreased striatal ^{18}F-fluoro-L-dopa uptake, observed using PET[18], and considerable loss of pigmented neurons in the substantia nigra.

In primates, administration of MPTP by stereotaxic application in the striatum, intra-carotid injections or repeated intravenous injection over 5–10 days[19–21] generally induces a substantial DA depletion resulting in a severe akineto-rigid PD syndrome (often requiring drug therapy) within weeks after administration of the neurotoxin. In contrast, repeated low-dose administration of MPTP over a longer period of time (up to 19 months) increases the selectivity of the neurotoxin for specific subpopulations of DA neurons, more accurately reproducing the pattern of neuropathological and neurochemical alterations observed in idiopathic PD[22,23]. In this chronic administration model, and in idiopathic PD[5,24], signs develop gradually, and after these signs appear they do not spontaneously recover as reported in some acute MPTP models[25]. This animal model therefore represents a stable parkinsonian syndrome, which is necessary for the exploration of long-term functional changes and experimental therapies.

The ability of MRS to sensitively measure neurochemicals in brain volumes less than 1 ml provides a unique 'window' into neurodegenerative processes. MRS is especially useful because it allows quantification of different chemicals in a single study, which can be repeated many times. Chemicals quantifiable in proton MRS include N-acetyl aspartate (NAA), a correlate marker for healthy mature neurons[26,27]. Thus, MRS has been used to study neuronal loss, using NAA as a marker[28–31]. Loss of NAA may not always correlate with the final destruction of

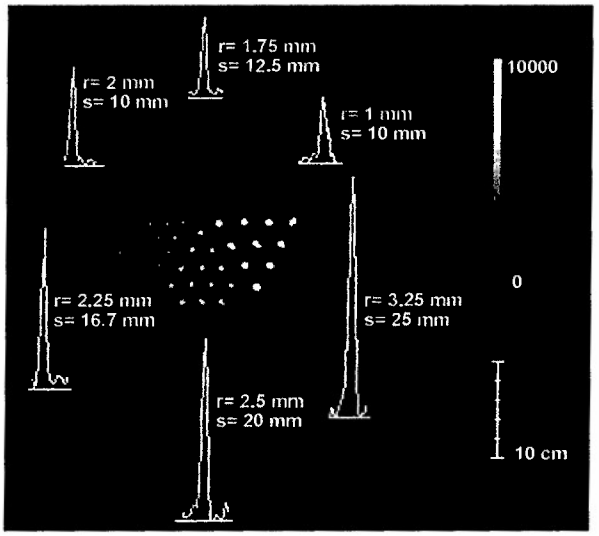
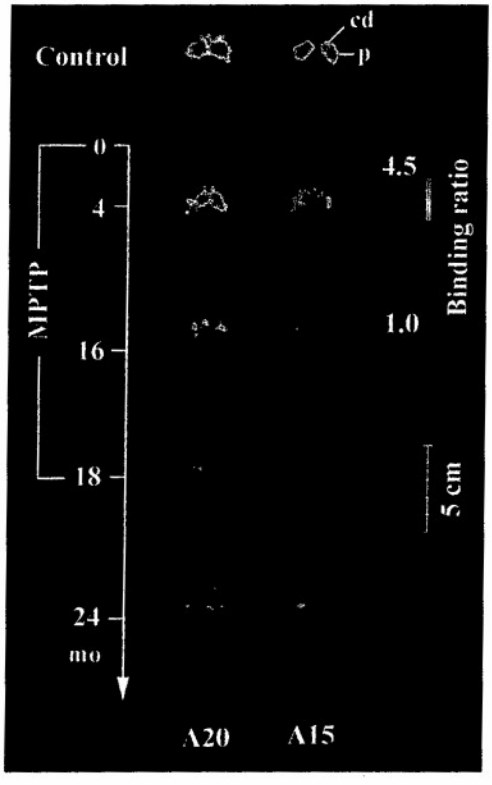


Fig. 1 PCR-I with ^{18}F-labeled water in a 'Derenzo-phantom'. PCR-I is a high-resolution brain imaging device; the phantom is a solid plastic disk with six sectors of holes each of a different radius (r) and separations (s). There is uniform distribution of radioactivity in all the sectors, with clearly separated images even for holes 2.0 mm in diameter with 10-mm separation. Spectra next to each sector describe measured count distribution in a single hole in each sector corresponding a volume of $3.14 \times (r)^2 \times 5$ mm^3 (the thickness of the slice is 5 mm). Scale bar represents 10 cm, with each division being 2 cm.

neurons, but to some degree may reflect their health[32,33]. In addition to NAA, substances such as lactate, glutamate, creatine, choline and myo-inositol provide a view of the progression of neurodegeneration; for example, in gliosis, glial cells have a concentration of cholines (trimethylamines) twice that of neurons[27]. Elevated choline concentrations are also found in conditions involving the proliferation of pathological forms of glial cells such as gliomas[7,8]. The main limitation in using MRS is its relative insensitivity compared to PET, because of the low signal obtained per molecule. NAA, the most prominent molecule in a brain proton spectrum, has an approximate concentration of 8–10 mM in the brain. Even at this concentration, MRS yields a low signal-to-noise ratio, which leads to a relatively low spatial resolution. Recent developments in PET instrument design have greatly improved the performance of PET[34,35]. Theoretically, the resolution of PET is limited by three factors: positron range, small angle deviation, and the sampling achieved by the detectors. For these experiments, positron emission tomography studies were done using a PET scanning system (PCR-I) equipped with one ring of 360 BGO (bismuth germanate) detectors and a computer controlled imaging table[36]. Here we have studied the long-term physiological changes after MPTP-induced neurotoxicity using PET and MRS techniques, in a primate model of PD.



Functional PET studies

Using a specially adapted PET scanning system (Fig. 1), we investigated chronic neurodegenerative processes over 3 years in a Parkinson disease model in five cynomolgus monkeys (Macaca fascicularis). We used carbon-11-labeled 2β-carbomethoxy-3β-(4-fluorophenyl) tropane (^{11}C-CFT, or WIN 35,428) as a tracer for visualizing dopamine re-uptake sites located on presynaptic dopamine terminals in experimental animals. We compared regional accumulation of ^{11}C-CFT in the striatum at two different coronal brain levels (A20 and A15 from the stereotaxic zero) with its accumulation in the cerebellum in the weeks before, during and after administration of MPTP; this treatment produces a parkinsonian brain degeneration of the dopamine system (Fig. 2). The striatal-to-cerebellar ratio of the ^{11}C-CFT accumulation was 4.5 in the pre-MPTP study and declined with the onset of MPTP administration. Spontaneous locomotor activity decreases in parallel with the decline of the ^{11}C-CFT uptake[23]; however, overt Parkinsonian signs appear only after locomotor activity and the ^{11}C-CFT uptake rate decline to about 30% of their pre-MPTP values[23]. Here the putaminal binding potential of ^{11}C-CFT continues to decline 5–8 months after termination of MPTP administration (Fig. 2) and remains at this level for 2 years after MPTP treatment (Fig. 3). Similarly, the ^{11}C-CFT levels in caudate continued to decline from 55% when MPTP treatment was stopped (Fig. 2) to 21 ± 9% 5–8 months after its termination, and remained at this level for 2 years (Fig. 3). Thus, functional degeneration of DA terminals continues for approximately 5–8 months after MPTP treatment ends and then does not spontaneously recover. During MPTP administration, ^{11}C-CFT accumulation decreased at a faster rate in putamen than in caudate (as seen in PD)(Figs. 2 and 3), indicating that DA terminals are more sensitive to MPTP in the putamen than in the caudate.

MRS studies during neural degeneration

We used ^{1}H water-suppressed MRS to measure biochemical changes in the striatum during MPTP-induced neurodegenera-

Fig. 2 A long-term follow-up study of comparative distribution of ^{11}C-CFT in a primate Parkinson disease model. Two representative coronal brain levels (A20 and A15) are presented before (0), during (4,16,18) and 6 months after (24) MPTP treatment. Images are normalized to cerebellar activity and represent distribution of specific to nonspecific binding of ^{11}C-CFT in the brain 60–62 min after administration of the labeled ligand.

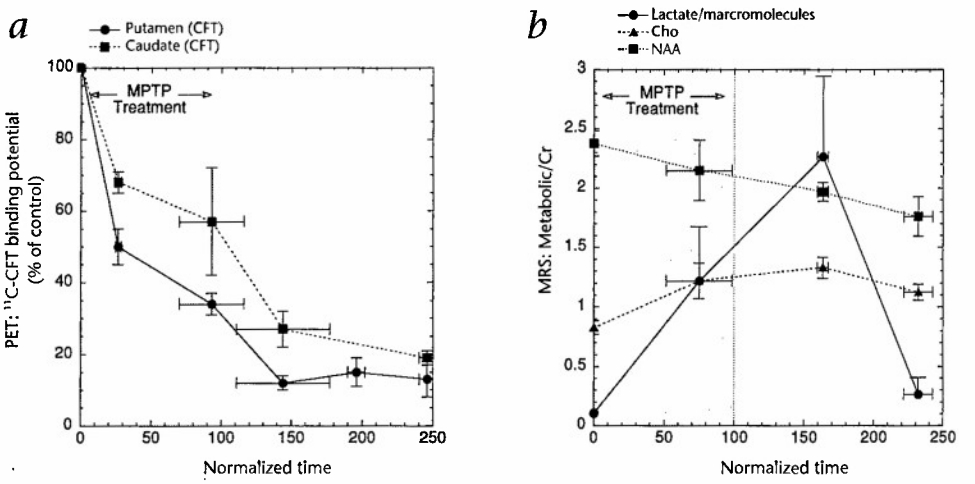


Fig. 3 PET studies of ^{11}C-CFT binding (***a***) and MRS studies of striatal biochemistry (***b***) before, during and after MPTP-induced neurotoxicity. There were irreversible changes of ^{11}C-CFT binding, choline and N-acetylaspartate concentration, as well as a 23-fold increase in peaks corresponding to lactate and macromolecule concentration that was reversible. Normalized time scale (horizontal axis) is obtained based on the response to MPTP-induced neurotoxicity in individual monkeys (as in patients, susceptibility varies). When the monkey showed overt parkinsonian symptoms, MPTP was terminated. The time of the MPTP treatment was normalized to 100, and the follow-up period was also normalized for each animal according this scale. The control value of the binding potential (k_3/k_4) was normalized to 100 and all the follow-up values were also normalized using this scale. The average follow-up time post MPTP was 2 years and the average value of the binding potential in control studies was 4.6–5.6 in putamen and 4.8 –6.6 in caudate region of the striatum.

tive processes. Complementary studies of DA re-uptake sites by PET and neurochemical changes by MRS are shown before MPTP treatment and 2 months after the last MPTP administration (Fig. 4). MPTP induced elevation of lactate/macromolecules and choline peaks (Figs. 3 and 4). Even as much as 10 months after termination of MPTP-induced neurotoxicity, the elevation in lactate/macromolecular peak was 23-fold ± 7-fold (Fig. 3). The choline/creatine (Cho/Cr) ratio in control monkeys was 0.83 ± 0.06 (Fig. 4), whereas it was 1.30 ± 0.15 in the 8–10 months after MPTP-induced neurotoxicity (Figs. 3 and 4). The NAA/Cr ratio in the control monkeys had very high inter-animal reproducibility (2.38 ± 0.11). This ratio decreased slightly but significantly in MPTP-treated monkeys to 1.93 ± 0.21 ($P < 0.01$) in the striatum 8–10 months after termination of MPTP treatment. This finding may reflect that MPTP is mostly neurotoxic for dopaminergic neurons in the substantia nigra, with only transsynaptic anterograde degeneration of the striatum[37,38]. Our data also show that the changes in NAA and Cho persisted after MPTP-induced neurotoxicity (Fig. 3). Two years after MPTP treatment stopped, the increase of choline in treated monkeys was 38 ± 4 % of the control value, and the corresponding decrease of NAA was 26 ± 4 % (Fig. 3). In contrast, the changes in lactate /macromolecular signal are reversible; by 2 years after final MPTP administration, this value had returned to control (background) levels. At approximately the time the striatal level of DA reuptake sites (^{11}C-CFT) reached a minimum in PET studies, the lactate peak seen with MRS reached a maximum.

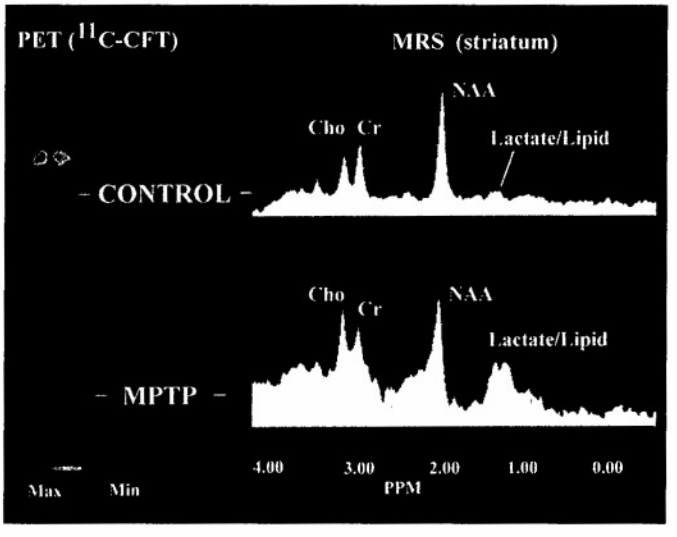


Fig. 4 PET and MRS studies of a monkey before any MPTP and 2 months after the last MPTP treatment. PET images (left) demonstrate that specific/nonspecific binding ratio of ^{11}C-CFT was considerably decreased after MPTP treatment (color coded by Max–Min bar at bottom). MRS (right) demonstrates a decreased NAA/Cr ratio, an elevated Cho/Cr ratio and an elevated lactate and macromolecule peak after MPTP treatment (TR/TE 2000/272 ms; PRESS).

Discussion

These experiments demonstrate, through the combined use of PET and MRS methods, a dynamic and specific neurochemical pattern of long-term neurodegenerative changes in the primate striatum after DA loss similar to that of PD. The physiological changes characterized by this combined PET/MRS approach provide data for a comprehensive *in vivo* analysis of the ongoing biological processes occurring after selective neural degeneration.

In animal models[22,23] and in humans[39], ^{11}C-CFT is a useful ligand to monitor DA terminal degeneration by PET scanning[23]. CFT was the first ligand to demonstrate a loss of DA fiber density equivalent to the loss of DA in human post-mortem Parkinson-diseased brains[40]. ^{11}C-CFT binding also correlates with motor signs in the MPTP primate model of Parkinson disease[22]; these observations have been verified in a larger series of primates[23] and are analogous with findings in early Parkinson disease in humans[40].

Here we studied ^{11}C-CFT levels and biochemical parameters in the striatum of each monkey for about 2 years after the monkey developed overt parkinsonian signs (at which time MPTP treatment was terminated). These data show persistent long-term physiological changes in striatal CFT binding and MRS-identified levels of choline and NAA. The changes in NAA and

choline levels were moderate and are consistent with an interpretation that MPTP-induced neuronal loss is mostly in the substantia nigra and that transsynaptic anterograde degeneration is in striatum[37,41]. MRS studies in patients with idiopathic Parkinson disease show few changes in striatal NAA or choline, but decreases of NAA and increase of choline are seen in some forms of parkinsonism[42,43].

We noted large changes in the MR spectral region between 1 ppm and 1.5 ppm (corresponding to lactate and macromolecules). Before the oxidative stress induced by MPTP, the intensity of this spectral band in the striatum was at background level, but with MPTP treatment, several large selective increases in striatal signal intensity were observed. First, there was a large increase of intensity at 1.33 ppm consistent with elevations in lactate (Fig. 3). After termination of MPTP treatment, there were even larger increases in signals at both 1.0 and 1.3–1.5 ppm. These later changes may reflect ongoing oxidative stress caused by physiological adaptive changes in function of the striatum. The presence of large amounts of mobile lipids acutely after MPTP treatment provides evidence for neuronal membrane breakdown possibly caused by lipid peroxidation or cell death mediated through cellular respiratory-chain inhibition[10,38]. However, the molecular species involved have not yet been specifically identified[10,38]. Detailed histological analysis of the striatum, however, indicates very little macrophage infiltration or gliosis in the MPTP-treated striatum in this progressive MPTP-induced degeneration[20,22]. Nonetheless, minor local striatal neuronal loss around large blood vessels and arterioles has been observed (O.I. and N.K. Kowall, unpublished observation), probably a consequence of direct MPTP-induced neuronal degeneration and mild gliosis from high toxin levels next to blood vessels (from intravenous administration of MPTP). The changes in the lactate and macromolecular peaks are reversible, however, and return to baseline 2 years after termination of MPTP administration.

These dynamic neurochemical shifts that occur several years after the neurotoxic event may relate to important physiological and pathological processes. For example, the signs of PD are not discernable in a patient until there is a 60–80% decrease in striatal dopamine levels. This in itself indicates fundamental adaptive physiological processes that maintain striatal function despite considerable degeneration of one transmitter system. Beyond this critical threshold, PD unfolds in a movement disorder that can, at least initially, be reversed by DA drug replacement therapy. Because the results of the MPTP treatment used here closely resemble the DA degeneration seen in PD, the movement disorder in this primate model also develops at the critical threshold of DA loss[22,23]. The dynamic and persistent physiological changes seen here using PET and MRS may therefore reflect similar adaptive striatal responses to those occurring in PD. Furthermore, the oxidative stress seen years after the neurotoxic events leading to DA loss indicate that the striatal neuronal circuitry may be compromised and at risk for subsequent structural and pathological processes. Future investigations should determine if such physiological stress of the caudate-putamen also occurs after other types of neurodegenerative events, or after long-term pharmacologically induced changes of the DA system[44].

These data indicate that the structural and neurochemical changes after a DA neurotoxic event are dynamic and complex, and continue to develop long after the neurodegenerative stimulus has stopped and PD signs develop. The characterization of these physiological changes may provide insights and a time frame for new therapeutic interventions in PD.

Methods

Primate model. The behavioral model of PD in cynomolgus monkeys (Macaca fascicularis) was produced by the chronic administration (0.6 mg/kg intravenously, every 2 weeks until behavioral stability) of the mitochondrial complex 1 inhibitor 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine[23] (MPTP). Spontaneous locomotor activity was quantified by continuous monitoring with four pairs of infrared motion detectors. Additional video recording and assessment was done monthly. Hypokinesia (decreased frequency of spontaneous movement), bradykinesia (slowness of movement) and tremor were rated by two independent observers to generate a clinical score (0–12), as reported[23]. Animals used in this study were maintained according to the guidelines of the Committee on Animals of the Harvard Medical School and Massachusetts General Hospital and those of the *Guide for Care and Use of Laboratory Animals* of the Institute of Laboratory Animal Resources, National Research Council, Department of Health, Education and Welfare.

PET techniques. The resolution of PCR-I for a point source at the center is 4.5 mm, and the sensitivity is 46,000 counts per s for a source 20 cm in diameter with a concentration of 1 μCi/ml. The overall detection efficiency of photons is 64% of the theoretical maximum for a plane thickness corresponding to the 2-cm-high detectors. A plane thickness of 5 mm (as used in this study) is obtained by limiting the effective height of detectors with cylindrical collimators, and it corresponds to a volume resolution of 0.08 ml. The resolving time of the PCR-I is 6 ns (FWHM).

We used a 'Derenzo-phantom' initially, with ^{18}F-labeled water as a radioactive tracer (Fig. 1). The phantom is a solid plastic disk with six sectors of holes of different diameters and separations. All the holes have the same length (25 mm). The smallest holes have a diameter of 2.0 mm and the separation between the midpoints of the holes is 10 mm. The largest holes have a diameter of 6.25 mm and the separation between the midpoints of these holes is 25 mm. Using the PCR-I, it is possible to image objects 2 mm in size separated by 1 cm (Fig. 1).

The synthesis of ^{11}C-CFT involves direct ^{11}C-methyl iodide methylation of 2β-carbomethoxy-3β-(-4-fluorophenyl)tropane (WIN 35,428; prepared by Organix, Woburn, Massachusetts) as published[45]. For PET imaging, monkeys were anaesthetized with 30mg/kg ketamine and 3mg/kg xylazine (initial dose, intramuscularly), and anesthesia was maintained with half this dose as needed. The femoral artery and vein were catheterized for collection of blood samples and injection of labeled ligand. The monkey was placed in the imaging position and the head was adjusted in a stereotaxic headholder with the earbar as a reference plane. Interior orbital supports ensure that images are acquired in pseudocoronal plane perpendicular to the orbito–meatal line. This allows superposition of data from MRI and MRS studies. After administration of labeled ligand (5 mCi; specific activity 600–1000 mCi/μmol) into the femoral vein, imaging data were collected 'stepwise' on seven coronal levels: A30 (that is, 30 mm anterior from the earbar), A25, A20, A15, A10, P5 (that is, 5 mm posterior from the earbar) and P10. The initial acquisition time per image was 15 s; it was subsequently increased to 60 s with the total imaging time being 90 min. Eighteen arterial blood samples of 0.1 ml each were drawn to monitor the decrease of radioactivity, starting a frequency of 15 s and ending with a frequency 15 min. In addition, six arterial samples were collected for HPLC analyses of metabolites of labeled ligand. Calibration of the positron tomograph was done for each study session, using the cylindrical plastic phantom (diam. 6 cm) and ^{18}F-labeled water. Cross-calibration with a gamma counter (Cobra Auto-gamma; Packard, Downers Grove, Illinois) was also done using ^{18}F-labeled water. Plasma data were corrected for counting efficiency, calibration factor and measured metabolites, and percent activity of the injected dose and ligand concentration were calculated. Imaging data were corrected for uniformity, sensitivity, attenuation, decay and collection time. PET images were reconstructed using Hanning weighted convolution backprojection[46]. Regions of interest (including left and right caudate and putamen, frontal cortex and cerebellum) were outlined from anatomical representations on the screen, and activity per unit volume, percent activity of the injected dose and ligand concentration were calculated. Data were analyzed using a three-compartmental model[39] and

SAAM program[47]. Plasma data were corrected for metabolites using an experimental two exponential correction function; $f(t) = 0.709 \times \exp(-0.108 \times t) + 0.286 \times \exp(-0.014 \times t)$. Binding potential was calculated as a ratio of transportation coefficients (k_3/k_4) into (k_3) and from (k_4) the area of interest (caudate or putamen).

MRS techniques. Monkeys were scanned on a GE 1.5T Sigma scanner (General Electric, Milwaukee, Wisconsin) using a saddle coil 15 cm in diameter. Monkeys were anesthesized with a dose of a mixture of 30 mg/kg ketamine and 3mg/kg xylazine. In the neurochemical analysis[30], single voxel spectra were recorded from striatum in the monkeys using a standard point resolved spectroscopy (PRESS) sequence (TR/TE = 2000/272 ms and 2000/136 ms, 2-kHz sweep width) with presaturation of the water using three chemical shift selective suppression (CHESS) pulses. The voxels were prescribed from a coronal plane and were optimized to cover both caudate and putamen. The voxel sizes ranged from a minimum of $8 \times 8 \times 9$ mm^3 (0.6 cm^3) to a maximum of $1 \times 1 \times 1$ cm^3. Data were analyzed using the NMR1 (New Methods Research, Syracuse New York) software package. After apodization with an exponential multiplication corresponding to a 1–2-Hz line-broadening and Fourier transformation, the major metabolites[30] were integrated in the frequency domain using curve fitting and assuming mixed Lorenzian-Gaussian lineshapes. Metabolite intensities were normalized relative to the phosphocreatine/creatine peak at 3.03 ppm as the denominator.

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Dopamine imaging markers and predictive mathematical models for progressive degeneration in Parkinson's disease

A.L. Brownell[1,3], B.G. Jenkins[1], O. Isacson[2,3]

[1] *Department of Radiology and* [2] *Neurology, Massachusetts General Hospital, and Program in Neuroscience, Harvard Medical School, Boston, MA 02114;* [3] *Neuroregeneration Laboratories, McLean Hospital, Belmont, MA 02178, USA*

Summary – We conducted PET imaging studies of modulation of dopamine transporter function and MRS studies of neurochemicals in idiopathic primate Parkinson's disease (PD) model induced by long-term, low-dose administration of MPTP. MR spectra showed striking similarities of the control spectrum of the primate and human striatum as well as MPTP-treated primate (six months after cessation of MPTP), and Parkinson's disease patient striatum (68 year old male; Hoehn-Yahr scale II; 510 mg/d L-DOPA). The choline/creatine ratio was similar in the MPTP model and human parkinsonism, suggesting a possible glial abnormality. The progressive degeneration of dopamine re-uptake sites observed in our PD model can be expressed by a time dependent exponential equation $N(t) = N_0 \exp(-(0.072 \pm 0.016)\, t)$, where N_0 represents intact entities (dopamine re-uptake sites before MPTP) and 0.072 per month is the rate of degeneration. When the signs of PD appear, N(t) is about 0.3–0.4 times N_0. Interestingly, this biological degenerative phenomena has similar progression to that observed in cell survival theory. According to this theory and calculated degeneration rate, predictive models can be produced for regeneration and protective treatments. © 1999 Elsevier, Paris

dopamine transporters / L-DOPA / MPTP / MRS / Parkinson's disease / PET

Parkinson's disease (PD) is one of the most common neurologic disorders. It is estimated that about 1 million Americans are affected by Parkinson's disease and about 40,000 new patients are diagnosed every year. Hypotheses of the etiology of PD are focused on possible genetic links (such as *α-synuclein*) and on the potential contribution of toxins (exogeneous and/or endogenous) [78, 79] and their potential interaction with genetic components [15]. At the cellular level PD is characterized by severe depletion of DA neurons and associated loss of synapses in the basal ganglia.

PD is diagnosed clinically based on the cardinal signs: tremor, rigidity, bradykinesia and postural instability [66]. Improved understanding of the pathophysiologic mechanism underlying parkinsonian signs and symptoms [70], as well as refinement of methods and techniques in neuroradiology, neurosurgery and neurophysiology, have stimulated the recent interest in developing therapeutic techniques. Investigations of MPTP (1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine)-induced parkinsonism in non-human primates have led to the hypothesis that dopamine deficiency in striatum leads to unbalanced activity from subthalamic nucleus into globus pallidus, resulting excessive inhibitory outflow (increased and synchronized spontaneous firing rate) from the internal segment of the globus pallidus [25]. This suppresses the motor thalamus which reduces activation of the cerebral cortex motor system, resulting in deficiency of movement [6, 25]. To interrupt this basal ganglia-motor system circuitry; three different therapeutic modalities are used, namely pharmacological therapy [52, 72, 80], fetal cell transplantation [28, 29, 51, 59], and surgical procedures such as pallidotomy [30, 36], thalamotomy [46] and chronic thalamic high frequency stimulation [4].

A recent extensive PD twin study indicates that physiological and toxic factors play roles in causing typical PD as humans age [79]. This progressive decline of dopamine (DA) terminals seen in idiopathic PD can be closely modeled in the non-human primate *Macaca fascicularis* by a low-dose exposure of the mitochondrial toxin, MPTP [8, 42, 81].

Developing radiopharmaceuticals for detection of dopamine terminals has been a major challenge for pharmacological research. Since autoradiographic studies of using cocaine analogs to label dopamine transporters were introduced [49], tropane derivatives have been widely used in PET imaging studies of

Parkinson's disease and drug abuse [35, 40, 52]. The latest developments, however, involve specific and sensitive cocaine analogs labeled with technetium-99m or iodine-123, used in single photon emission tomography studies of dopaminergic system [7, 22, 38, 53, 64].

The ability to observe both physiology and function in small areas within the brain is now possible with high resolution PET and MR imaging techniques [11, 16, 47]. The potential use of positron emission tomography (PET) as a research tool in movement disorders has been demonstrated in studies of brain dopamine function [74] and glucose metabolism associated with movement disorders [1, 43, 71]. Recently, high resolution PET imaging has been widely used in studies with animal models of Parkinson's disease [8-10, 18, 19, 42, 48, 81]. In addition, advances in receptor studies [10, 32, 42], and magnetic resonance spectroscopy of neurodegeneration [8, 24, 39, 44, 47], provide specific functional neurochemical information.

Our earlier work indicated; (1) that a stable Parkinson-like disease appears after chronic administration of a neurotoxin, MPTP; (2) that progressive dopaminergic fiber loss can be detected by positron emission tomography (PET) using carbon-11 labeled 2β-carbomethoxy-3β-(4-fluorophenyl) tropane (^{11}C-WIN 35,428 or ^{11}C-CFT) to label dopamine reuptake sites [42, 81]; and, (3) that progressive physiological changes of neurochemicals occur as observed with MRS and PET [8]. In the present article, we compare imaging characteristics of ^{11}C-CFT with those of ^{18}F-L-6-fluorodopa, and show that by using ^{11}C-CFT the progressive degeneration of dopamine terminals can be mathematically modeled to determine the rate of degeneration and predict the time of onset of PD signs.

MATERIALS AND METHODS

Study design

Longitudinal PET and MRS imaging studies were carried out in six MPTP-treated primates *(Macaca fascicularis)* to follow the progression of the MPTP-induced degeneration. These primates served as their own controls in studies prior MPTP. Control studies with MRS included four additional primates *(table I)*. Comparison of MRS primate data was done with one Parkinson's disease patient (68 year old male; Hoehn-Yarn scale II, 510 mg/d L-DOPA) and an aged matched normal volunteer.

MPTP-lesion in primates

A slow neurotoxic lesion of dopaminergic cells located in the substantia nigra and in the ventral tegmental area was obtained by repetitive administration of MPTP dissolved in saline and immediately administered intravenously to primates (0.6 mg/kg i.v., every two weeks until behavioral stability) under light anesthesia (ketamine, 5 mg/kg i.m.), as previously described [81].

Table I. Striatal neurochemical changes in primates 0.5–2 years after cessation of MPTP treatment.

Metabolite Ratio	*Controls (n = 10)*	*MPTP (n = 6)*
NAA/Cr (range)	2.38 ± 0.11 (2.3–2.5)	2.09 ± 0.29* (1.7–2.5)
Cho/Cr (range)	0.83 ± 0.06 (0.8–0.9)	1.20 ± 0.15*** (1.0–1.4)

Unpaired Student's t test values for difference from control: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

In this chronic model, behavioral signs developed gradually over 9–14 months, progressing from bradykinesia to akinesia in all limbs. Tremor also occurred as the last PD sign. These signs did not spontaneously recove, in contrast to acutely induced MPTP-PD models [20, 31, 54].

PET imaging studies of dopamine transporters

Instrumentation

Positron emission tomography studies were carried out with PET scanning system, PCR-I [11], as earlier described [8].

Labeling of radiopharmaceuticals

Radiolabeling of ^{11}C-CFT was published earlier [10] and L-6-^{18}F-fluorodopa was prepared according to the fluorodemercuration method [62].

Experimental procedures

For PET imaging, animals were anaesthetized with ketamine/xylazine (30/3 mg/kg i.m.) initial dose and anesthesia was maintained with half a dose hourly injections as needed. Catheterization of the femoral artery and vein was used for collection of blood samples and injection of labeled ligand. The animal was placed in the imaging position, and the head was adjusted into a stereotactic headholder with the earbar at the origin. Interior orbital supports ensure that images were acquired in pseudocoronal plane perpendicular to the orbito-meatal line. This allows superposition of data from MRI and MRS studies. After the injection of labeled ligand, ^{11}C-CFT or ^{18}F-L-6-fluorodopa (5mCi, specific activity 600–1,000 mCi/μmol) into the femoral vein, imaging data were collected stepwise on seven levels (A30 (30 mm anterior from the origin), A25, A20, A15, A10, P5 (5 mm posterior from the origin) and P10) initially using 15 s frames. The frame time was subsequently increased to

60 s, the total imaging time being 90 min for ^{11}C-CFT and 120 min for ^{18}F-L-6-fluorodopa. While imaging with ^{11}C-CFT, 18 arterial blood samples of 0.1 mL were collected at different time points starting from 10 s frequency and ending with 15 min frequency to monitor the decrease in radioactivity. In addition three arterial blood samples were collected for HPLC analyses of metabolites of labeled ligand.

Calibration of the positron tomograph was performed in each study session using a cylindrical plastic phantom (diameter 6 cm) and ^{18}F-solution. Cross calibration with a gamma counter (Packard Cobra Auto-gamma, Downers Grove, IL, USA) was carried out using the same solution. Imaging data were corrected for uniformity, sensitivity, attenuation, decay and collection time. PET images were reconstructed using Hanning weighted convolution back-projection [13]. Regions of interest including left and right caudate and putamen, frontal, parietal and temporal cortex, thalamus and cerebellum were drawn on each level and activity per unit volume, percent activity of injected dose, and ligand concentration were calculated. Plasma data were corrected for counting efficiency, calibration factor and measured metabolites of ^{11}C-CFT and percent activity of injected dose and ligand concentration were calculated. Plasma data was used as an input function in the kinetic modeling.

Receptor studies with ^{11}C-labeled CFT

The kinetic behavior of ^{11}C-CFT was studied with a three compartmental model approach [77]. In the three compartmental model, the first compartment is the plasma pool, the second is the exchangeable tracer pool including free and nonspecifically bound ligand in the brain, and the third compartment is a trapped tracer pool including bound ligand in the brain. The exchangeable tracer pool contains ligand but no receptors and the third compartment includes all the receptors, partly or totally occupied by ligands. The kinetic parameters k_3 and k_4 describe the binding to and dissociation from receptors.

The transfer coefficients k_1–k_4 were mathematically resolved using the SAAM II program [26]. For stabilization of the k values the fitting procedure was performed using three steps. Since cerebellum does not have specific receptor binding or it is negligible, fitting was done in the cerebellum data letting all the k-values float. Briefly, with estimates for the initial conditions for the k-values, the differential equations were integrated using an adaptable fourth order Runge-Kutta method with suitable accuracy (tolerance 1^{e-7}). Iterations continued until sufficient convergence was achieved for the system parameters (k_1–k_4). The ratio k_1/k_2 was calculated. In further iterations of the striatal data the fixed ratio (k_1/k_2) was used as a constraint to reach parameter optimization. Regional binding parameters k_3/k_4 were calculated for each study.

Comparison of imaging characteristics of ^{11}C-CFT and ^{18}F-L-6-fluorodopa

Comparison of imaging characteristics of ^{11}C-CFT and ^{18}F-L-6-fluorodopa was based on obtained contrast in striatum compared to cerebellum. The difference of the striatal and cerebral accumulation of radioactivity was fitted into gamma variate function and the maximum value was divided by the value of the cerebral activity at that time point.

Modeling of progressive degeneration

To analyze MPTP-induced progressive degeneration, values of striatal binding potentials of ^{11}C-CFT at different time points during the MPTP-administrations (time = 0 when MPTP-administration was started) were fitted into an exponential function; $N(t) = N_0(t = 0) \exp(-k\,t)$. N_0 denotes binding potential in the intact dopamine terminals or arbitrary estimate of the intact dopamine terminals, N(t) is the corresponding value after degeneration of time (t) and k is a rate of degeneration.

MRS studies of neurochemicals

For these studies, we utilized single voxel spectroscopy of the basal ganglia. We chose voxels centered in the striatum for both monkeys and PD patients. Voxels were between 0.5–1 cm^3 in the monkey brain and between 3–5 cm^3 in the human brain. Water suppression was performed using CHESS pulses and localization by a standard PRESS-type sequence with TR/TE of either 2000/272 or 2000/136 ms. Spectra were processed using the NMR1 program (NMRI, Syracuse, NY), by curve fitting the entire spectrum and integrating the areas of the major metabolites. Integrals were then normalized to the creatine peak at 3.03 ppm (Cr) as a standard.

Metabolite quantification

We found NAA/Cr ratios to be reliable quantitative indicators of neurodegeneration. This reliability was indicated by the large differences noted between the MPTP-lesioned animals. In the case of single voxel spectroscopy we used a fully relaxed non-water suppressed spectrum from the voxel. This provides a constant internal reference for a metabolite/water ratio even if, due to metabolite T1 and T2 errors, absolute concentrations remain elusive. The stan-

dard deviations in this technique were very small, and allowed to make direct inter-animal comparisons.

Characterization of the elevated lipid/lactate peaks were performed using multiple TE values to characterize the coupling constants and double quantum filtration to estimate how much of the intensity is due to lactate. Due to the relatively shorter T1 values of lipids, we used inversion recovery PRESS spectra with variable TI values to characterize the lipid T1s in order to estimate the concentrations. In addition, we have implemented a STEAM sequence with the capability getting TE's down to 6 ms. This enables quantitative measurements of the lipid and macromolecular components when combined with the inversion recovery experiments.

RESULTS

Figure 1 shows ^{11}C-CFT and ^{18}F-L-6-fluorodopa distribution in the same control primate. Sixty minutes before the ^{18}F-L-6-fluorodopa injection the primate was pretreated with carbidopa (5 mg/kg) to reduce peripheral metabolism. These images show the striking specificity of ^{11}C-CFT to image striatal function. The contrast of striatal binding using ^{11}C-CFT was 3.25 ± 0.56 and correspondingly 1.67 ± 0.23 using ^{18}F-L-6-fluorodopa. Striatal data were averaged from putamen data of levels A20 and A15 from the left and right sides and caudate data of levels A25 and A20 from both sides. *Figure 2* shows relative ^{11}C-CFT binding distribution before and during MPTP administration in an asymptomatic and symptomatic stage. Three coronal brain

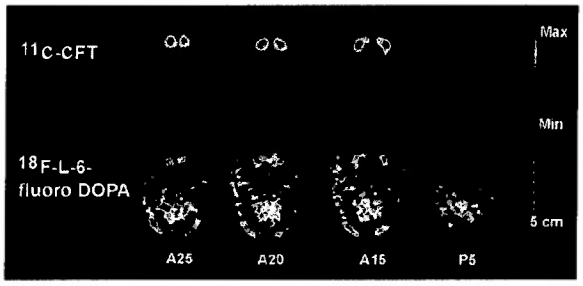

Figure 1. Color coded PET images showing ^{11}C-CFT and ^{18}F-L-6-fluorodopa accumulation in the same control primate brain. Sixty minutes before fluorodopa injection the animal was pretreated with carbidopa (5 mg/kg) to reduce peripheral dopamine metabolism. ^{11}C-CFT images are acquired 60–62 min after injection and ^{18}F-L-6-fluorodopa images 90–120 min after injection. Four images represent the brain levels A25, A20, A15 mm anterior and P5 mm posterior from the reference plain. After corrections for decay, acquisition time and injected activity the highest pixel value of the four ^{11}C-CFT images was normalized to 10,000 and the lowest to 0. All the ^{11}C-CFT images were normalized according to this scale. Correspondingly, after corrections the four ^{18}F-L-6-fluorodopa images were normalized similarly.

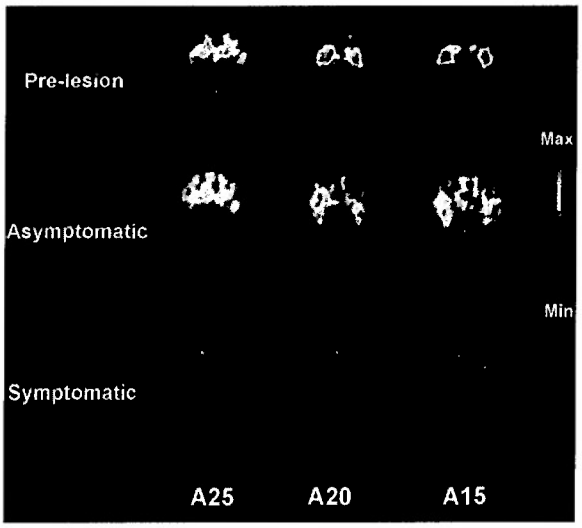

Figure 2. Color coded PET images showing relative ^{11}C-CFT binding in a monkey brain 60–62 min after injection. The three images represent the levels throughout caudate-putamen (A25, A20 A15 mm anterior of the reference plain) before MPTP treatment, after 3 MPTP injections, when the primate was asymptomatic and after 9 months of MPTP treatment, when the monkey was symptomatic. After corrections for decay, acquisition time and injected activity, the average count density was determined in cerebellum study and ^{11}C-CFT images of the three coronal brain levels were divided by this value on the pixel basis individually in each study. Finally, the highest pixel value in the nine images was normalized to 10, 000 and the lowest to 0. All the images were normalized according to this scale.

levels (A25, A20 and A15) through the striatum show that degeneration in putamen is more severe that in caudate. The progressive degeneration of dopamine reuptake sites observed in our primate PD model can be expressed by an exponential equation $N(t) = N_0 \exp(-k\,t)$, where N_0 represents intact entities (dopamine reuptake sites) and k represents the rate of progressive degeneration. *Figure 3* shows progressive degeneration observed in six primates during low-dose MPTP administrations. The exponential curve fitted to the calculated binding potential values is $N(t) = N_0 \exp(-(0.072 \pm 0.016)\,t)$ indicating that the rate of MPTP-induced degeneration is 0.072 per month. When signs of PD appeared, N(t) was about (0.3–0.4) N_0.

We have also investigated neurochemical changes with MRS in the same primates as imaged by PET using ^{11}C-CFT. Spectra from a control and typical MPTP-treated primate striatum (six months after cessation of MPTP therapy) is shown in *figure 4* with comparison to MR spectra of a parkinsonian patient (68 year old male, Hoehn-Yahr scale II, 510 mg/d L-DOPA) and an age matched control patient. Note the pronounced changes

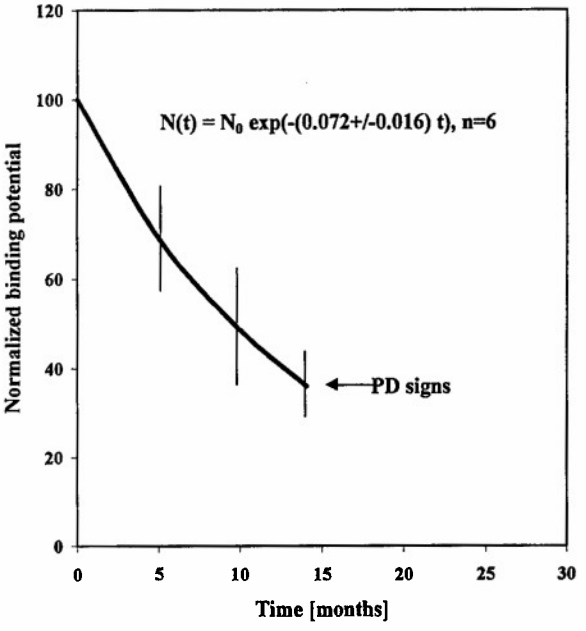

Figure 3. Model for the progressive degeneration and the appearance of parkinsonism in MPTP treated primates. Control value of the binding potential (before MPTP) was normalized individually to 100 and all the other values were normalized according this scale. (Raw data from [8].)

compared to the control striatum. Lactate and/or lipid peaks are visible in both the patient and the primate, but not in the controls. In all the primates studied (n = 6), the lactate/lipid peaks had disappeared after an additional eight months [8]. These data indicate an acute metabolic process which resolves after a period of time, and is consistent with the time course for macrophage infiltration. Unfortunately we were unable to collect enough data to completely assay the time course of changes in all the metabolites over time. Future studies will entail collection of more data to determine the complete spectroscopic time profile of evolution of the neurochemical changes.

In the MPTP model there is a significant decrease in NAA, which is larger than that seen in our PD patients (NAA/Cr = 2.09, n = 6 vs. 2.33 in PD patients, n = 23, B. Jenkins, personal communication). This is significant since our control human population had identical NAA/Cr levels to the primate controls (2.33 ± 0.46 in humans; n = 20 vs. 2.38 ± 0.11 in primates, n = 10, Jenkins, personal communication). Notably, in the MPTP monkeys there was a large increase in the Cho/Cr ratio, very similar to what is seen in our PD patients (Cho/Cr = 1.2). Choline may be reflective of gliosis as the choline concentration in glial cells is twice that in neurons or of macrophage activity. A quantitative summary of our primate results is shown in the *table I*.

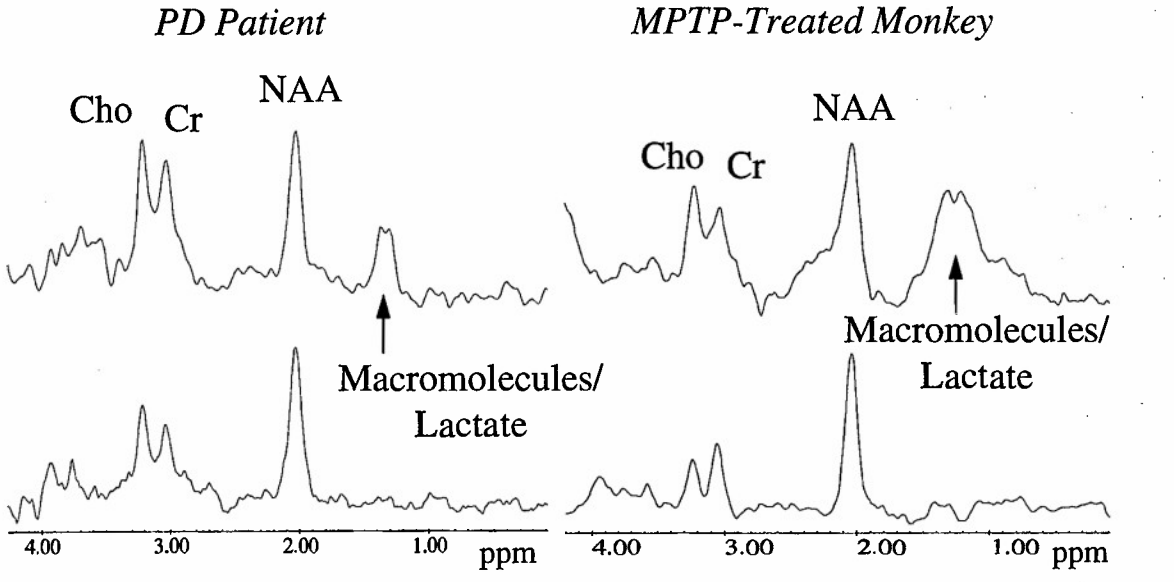

Figure 4. Striatal spectra from: Left) A PD patient (male; 68 years old; Hoehn-Yahr scale II; 510 mg/d L-DOPA), and an age-matched control. Right) An MPTP-treated monkey 6 months after cessation of MPTP-treatment and a control monkey. Major neurochemicals observed are indicated. Note the striking similarity of the control spectrum of the primate and human as well MPTP-treated primate and Parkinson's disease patient (TR/TE 2000/272ms; PRESS).

DISCUSSION

Realistic primate models that mimic the progressive changes of PD are of critical importance for developing neural therapeutic techniques. The optimal procedure for therapy-induced behavioral recovery observed in many clinical and experimental studies is still unclear.

Primate models of parkinsonism were developed using MPTP administered according to different protocols [12, 56]. Stereotaxic application of MPTP (or its active metabolite MPP+) in substantia nigra or in the striatum, as well as intra-carotid injections or repeated intravenous administration during 5–10 days [12, 45, 56], generally induces a marked dopamine depletion resulting in a severe akineto-rigid parkinsonian syndrome (often requiring drug therapy) within weeks following treatment. Such studies demonstrated that MPTP-induced behavioral, neurochemical and anatomical changes are analogous but not identical to alterations observed in parkinsonian patients [12, 21]. Acute protocols (toxicity induced over one to five days) of MPTP differ from idiopathic (PD) in several aspects: (1) pathologic changes in idiopathic PD extend beyond the substantia nigra [37]; whereas, the substantia nigra, and to a lesser extent the ventral tegmental area, are the regions primarily lesioned by MPTP toxicity; (2) acute MPTP-administration to non-human primates does not produce an uneven pattern of striatal dopamine loss described in idiopathic PD, with relative sparing of dopamine levels in the caudate nucleus compared to the putamen [21]; (3) acute MPTP toxicity in non-human primates also creates motor symptoms that may recover with time [20, 54]; (4) an acute administration protocol does not reproduce the chronic and slow degeneration of dopamine neurons that occurs in idiopathic PD. Recently, a less acute primate model of various stages of PD has been obtained by unilateral intra carotid infusion [48] combined with sequential systemic doses of MPTP [19]. In addition, a chronic model of PD has been introduced by using daily low dose systemic injections of MPTP for 22 days [5].

Following these principles, our studies involving chronic low-dose administration of MPTP [8], have clearly demonstrated that by repeated administration of the neurotoxin over a long period of time, it is possible to increase the selectivity of the neurotoxin for specific subpopulations of dopamine neurons, more accurately reproducing the pattern of neuropathological and neurochemical alterations observed in idiopathic PD.

Recent advances of in vivo receptor studies have resulted in the development of new receptor specific ligands [2, 23, 32, 33, 63] combined with advances in instrumentation for PET [3, 11, 16]. High resolution positron imaging yields accurate data over small regions inside the brain [9] that, combined with modeling of the ligand-receptor interaction, can provide valuable quantitative information about receptor behavior in different areas of the living brain.

Modeling of neuroreceptor kinetics has also been an active research area. Several methods have been proposed for estimating the binding parameters (B_{max}, maximum available receptor binding sites; K_D, dissociation constant; k_{on}, bimolecular association rate constant; and, k_{off}, dissociation rate). The choice of method depends on the particular properties of ligand-receptor interaction. In reversible binding, ligands dissociate from the receptor during the imaging period so that the maximum binding site density can be calculated from the equilibrium distribution [23]. In the case of irreversible binding, equilibrium is not achieved during the imaging period. The dopamine transporter specific ligand (^{11}C-CFT) has irreversible binding.

Two types of kinetic analysis are used to analyze PET data. The graphical method [60, 73] has been applied by our group to estimate the influx of ^{11}C-CFT to dopamine terminals [42], and by several groups in estimating the influx of L-6-^{18}F-fluorodopa [59, 67].The other method is based on general non-linear regression techniques [14, 61, 69, 77].

Research has demonstrated a significant correlation between depression of striatal ^{18}F-L-fluorodopa uptake of PD patients and their degree of locomotor disability. However, while the average putamen ^{18}F-L-dopa uptake in PD is reduced to 40% of normal, a 60% loss of nigra compacta cells and 80–90% loss of putaminal dopamine levels are found post-mortem in PD [34]. Therefore, striatal ^{18}F-L-fluorodopa uptake reflects metabolic and functional activity of nigro-striatal fibers, but may not accurately depict levels of endogenous striatal dopamine or anatomical depletion of dopamine terminals. A specific tracer for selective labeling of dopamine fibers would be preferable. Among various candidates for labeling dopaminergic fibers, specific ligands for dopamine re-uptake sites (dopamine transporter) such as ^{11}C-nomifensine, ^{11}C-cocaine or ^{18}F-GBR 13119 (1–((4–((18F)fluorophenyl) (phenyl)methoxy) ethyl)-4-(3-phenylpropyl) piperazine) have been used in PET studies [27, 50, 57]. In such PET studies, specific binding of the ligands to dopamine transporters were taken as a measure of monoaminergic nerve terminal density. However, using these ligands in vitro, binding assay showed only a 40% decrease of binding in the caudate nucleus and putamen of subjects with PD [65], while other measures for

dopaminergic terminals were reduced much more dramatically. Similar results have been obtained in vivo using ^{11}C-S-nomifensine as a PET tracer [58]. Again, the 40% decrease in dopamine re-uptake site density is strikingly different from the 90% decrease of dopamine levels measured post-mortem in parkinsonian putamen.

We have studied the imaging characteristics of carbon-11 labeled CFT in normal and MPTP-treated primates [10], and it has proved to be a very selective ligand [68] to monitor dopamine terminal degeneration having higher specificity than nomifensine or GBR analogues for the dopamine uptake complex [49]. Several observations suggest that CFT is a useful and specific marker for dopamine nerve terminal density: ^{11}C-CFT in vivo binding, as well as ^{3}H-CFT in vitro binding [49] in the non-human primate caudate nucleus, is highly specific for the dopamine transporter. ^{3}H-CFT binding was decreased in PD up to 95% depending on striatal region [49], and ^{3}H-CFT depletion in PD paralleled the dopamine depletion, with a more severe decrease in specific binding in the putamen than in the caudate nucleus [49].

Our group was the first to demonstrate that ^{11}C-CFT binding correlated with behavioral symptoms in a primate model of Parkinson's disease [42]. This has been verified in a larger series of primates [81], and also in early Parkinson's disease in humans [32]. After the earlier studies, several novel tropane derivatives have been introduced for imaging of dopamine transporters, mainly labeled with iodine-123 (altropane [64], beta-CIT [22], FP-CIT [7], PE21 [38] or technetium-99m (trodat) [53].

Figure 1 shows that the radiolabeled cocaine analog ligands e.g., ^{11}C-CFT provide better sensitivity and selectivity for imaging of the striatal dopamine system than radiolabeled L-dopa. *Figure 1* also demonstrates the effect of the increased active radiolabeled metabolites during imaging with ^{18}F-L-6-fluorodopa in blood rich areas in the head. ^{11}C-CFT used in PET imaging of MPTP treated monkeys demonstrate progressive DA terminal loss in caudate-putamen before and after appearance of PD signs. In addition, the observed MPTP-induced degeneration is more progressive in putamen than in caudate *(figure 2)*. Our new MRS studies illustrate lactate/lipid elevation in the striatum in both parkinsonian monkeys (post-MPTP) and in a typical case of a Parkinson's disease patient (68 year old male, Hoehn-Yahr scale II). This is consistent with previous studies [8], showing parallel increases in striatal lactate/lipid and continuous DA fiber (^{11}C-CFT) degeneration. In addition, the small decrease in NAA (12%) observed in the monkeys may also be reflective of the loss of dopamine terminals and striatal cell dendritic density.

Notably in the MPTP monkeys, there was a large increase in the Cho/Cr ratio which was almost identical to that of PD patients (Cho/Cr = 1.2). This is possibly an important physiological observation, since choline may reflect gliosis or magrophage activity. The various theories for neurodegeneration in PD includes one of loss of target-derived trophic support [17, 75, 76]. Glial cells typically provide both growth-factors and homeostatic support [75, 76, 82]. This finding deserves further investigation to determine if sub glial changes are a consequence or a primary cause of dopaminergic axonal degeneration in the caudate-putamen of PD.

Our data provides a basis for a mathematical model of degeneration of the DA system in PD. It is known that 60–70% degeneration in a dopaminergic system precedes the symptoms of PD. In our primate PD model, the remaining entities (dopamine re-uptake sites) were (0.3–0.4) of the original value when the PD signs appeared. Interestingly, this biological degenerative phenomena has similar progression to that formulated in cell survival theory in radiobiology concerning the effect of radiation in killing cells [41]. According to the formula, the number of survived cells (N_D) after radiation dose (D) is $N_D = N_0 \exp(-D/D_0)$, where N_0 is the number of cells before radiation and D_0 is the mean lethal dose of radiation. When the radiation dose (D) equals to the mean lethal dose (D_0), the function will get a form of $N_D/N_o = e^{-1} = 0.37$ and the number of survived cells is $0.37\ N_0$. Similarly, using the rate of degeneration (0.072 ± 0.016, *figure 3*), the calculated time to get PD signs is 13.9 ± 2.5 months in this MPTP-PD model, which is the same as was observed in experimental studies *(figure 3)*. With this theory and imaging studies of the dopaminergic system, a realistic estimate can be obtained of degeneration rate and the time when the patient will get PD symptoms.

CONCLUSION

^{11}C-CFT is a useful ligand for detection of PD-like progressive degeneration. Based on the decrease of ^{11}C-CFT binding, a rate of degeneration can be calculated and the time of onset of PD symptoms can be determined.

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CURRICULUM VITAE Updated 9/21/99

[redacted]

PII Redacted

EDUCATION:

University career and degrees

1979	Biochemistry, University-College of Kalmar.
1980	Medical School, University of Lund
1981	Research appointment, University of Lund, in the laboratory of Prof. A. Björklund.
1983	1st medical degree, Medical Bachelor, Medical School, University of Lund
1987	Doctor of Medicine (Dr Med Sc), University of Lund (May 1987) on thesis: "Neural grafting in an animal model of Huntington's disease"
1987	Fellow in Neurobiology, University of Cambridge, England
1989	*Docent* (Academic Title of Assoc. Professor) of Medical Neurobiology, University of Lund.

UNIVERSITY POSITIONS AND APPOINTMENTS:

1981-83	Teaching Assistant, Dept.of Histology, University of Lund, Sweden
1983-85	Research Assistant (Demonstrator), University of Lund, Sweden
1986-87	Lecturer & Research Associate, Dept. of Medical Cell Research, University of Lund, Sweden
1987-89	Research Fellow, Neuroscience, Dept. of Anatomy, University of Cambridge, England
1989-92	Asst. Professor of Neuroscience (Neurology), Harvard Medical School and Massachusetts General Hospital, Boston, MA, U.S.A.
1989-	Director of Neuroregeneration Laboratory, MRC, McLean Hospital
1992-	Associate Professor of Neuroscience (Neurology), Harvard Medical School and Massachusetts General Hospital
1993-	Associate Professor of Neuroscience, University of Massachusetts, Medical School

Personal scholarships and grant awards

1976-78	Nathorst's Scientific Foundation. Scholarship Award at Atlantic College, Wales, G.B.
1983	The Medical Faculty Award for thesis work in medicine, University of Lund, Sweden.
1987	The Fernstrom Foundation Scholarship Award 1987 for medical scientists
1987	The Swedish Physican's Society Award for studies on neurodegenerative diseases.
1987	The Royal Swedish Academy of Sciences. Lindahl's Award.
1987-88	European Neuroscience Association: Research Grant # 86/79
1987-89	Swedish Medical Research Council: Research Grant # K88-12P-08433

1989-90	NATO Grant, for studies on neurodegenerative disease. Research grant # CRG 890583
1990-91	NIH Program Project Award: Huntington's Disease Center, Massachusetts General Hospital and McLean Hospital.
1991-96	NIH: Neurological Science. Research Grant 1 R29 NS29178
1992-	NIH: Neurological Science. Research Grant 1 RO1 NS30064
1990-95	Training Grant in Neuroscience, Massachusetts General Hospital (P.I. Dr. Richard Masland)
1994-	Training Grant in Molecular Biology of Neurodegeneration, Harvard Medical School (P.I. Dr. Huntington Potter)
1994-	Training Grant in Clinical Neuroscience, McLean Hospital (P.I. Dr. Francine Benes)
1994-95	Milton Fund Award, Harvard University
1998-	USAMRMC Research Grant, DAMD-98-1-8618
1999-	USAMRMC Research Grant DAMD-99-1-9482
1999-	NIH: NINDS, Parkinson's Disease Center of Excellence, 1 P50 NS39793

Teaching and administrative experience

1981-83	Seminars and tutorials in Cell Biology, Histology and Neurobiology at the Medical Faculty, University of Lund, Sweden
1983-85	Lecturer in Neurobiology and Histology at the Medical Faculty, University of Lund.
1985-87	Lecturer and Assistant Director of Medical Neurobiology Course, Lecturer in Histology, Supervisor for research students in Medicine, Co-supervisor for 2 PhD students, University of Lund.
1987-89	V. Fellow, Jesus College, Cambridge, University of Cambridge, England. Supervisor for Medical Part II students, University of Cambridge, England.
1989-92	Asst. Professor of Neurology, Program of Neuroscience, Harvard Medical School, Boston, MA
1989-	Director of Neuroregeneration Laboratory, HMS, MRC, McLean Hospital, Belmont, MA: (Currently 5 post-doctoral, 5 pre-doctoral fellows and 3 support staff, lab space 2100 sq. feet) Neurology Research (Neuroanatomy), Massachusetts General Hospital, Boston, MA Principal Investigator, Harvard University, Primate Research Center, Southborough, MA (Currently lab space shared 1000 sq feet)
1990-	Post-doctoral Research Advisor for Residents in Neurology and Neurosurgery, MGH. Post-doctoral Research Advisor for Fellows in Neurobiology and Neurology, HMS. Faculty, Program of Neuroscience, Harvard Medical School. Senior Thesis Advisor, Harvard University, in Departments of Biochemistry, Biology and Psychology. Graduate Admission Committee, Program of Neuroscience, Harvard Medical School. Faculty, Neurobiology of Behavior course/program, McLean Hospital, Harvard Medical School Faculty, Lecturer, RUNN Course (Review and Update in Neurobiology for Neurosurgeons and Neurologists) Woods Hole, MA Faculty, Lecturer, Cold Spring Harbor Course: "Molecular Genetic Analysis of Diseases of the Nervous System"
1992-	Assoc. Professor of Neurology, Program of Neuroscience, Harvard Medical School, Boston, MA
1992	Course organizer: HMS Program of Neuroscience Graduate Course; "Paradigms to investigate neuronal health: what happens to neurons in neurodegenerative diseases".
1992	Faculty, Lecturer, Dept. of Neurology, HMS, MGH course: "Intensive Clinical and Basic

	Neuroscience Update", Boston.
1994	Massachusetts General Hospital, Scientific Integrity Course (faculty)
1995-	McLean Hospital, Clinical Neuroscience Training Program (faculty)
1995	Organizer, "Cellular and Molecular Treatments of Neurological Diseases" Scientific Conference, Cambridge, MA
1996	Gene Therapy: Principles and Practice (Genetics 208), Harvard Medical School "Strategies of gene therapy for dominant and recessive genetic, as well as non-hereditary, diseases" (lecture)
1996	Neurobiology of Disease Course, Harvard Medical School
1998	Co-director, "Anatomy and Physiology of Basal Ganglia Surgery" CME Course and Scientific Conference, Sarasota, FL
1998	Organizer, Second "Cellular and Molecular Treatments of Neurological Diseases" CME Course and Scientific Conference, Cambridge, MA
1999	Co-director, Second "Anatomy and Physiology of Basal Ganglia Surgery" CME Course and Scientific Conference, Sarasota, FL

Memberships

International Brain Research Organization (IBRO)
American Association for the Advancement of Science (AAAS)
Society for Neuroscience
European Neuroscience Association (ENA)
Boston Society of Neurology and Psychiatry
Huntington's Disease Society of America, Massachusetts Chapter
American Society for Experimental Neuropathology
World Federation of Neurology Huntington's Disease Research Group
American Society for Neural Transplantation and Repair (ASNTR)

Editorial Boards

Cell Transplantation
J. Neural Transplantation and Plasticity
J. Restorative Neurology and Neuroscience
Experimental Neurology

Membership on Advisory Committees

1992-	Veterans Administration Merit Review Board, USA
1992-	Internal Review Committee NIH Program Project grants
1993-	*Ad hoc* member ; Special Review Committes; National Institutes of Health (NINDS): Program Projects (Site-visit teams) and Clinical Research Centers (NIH)
1994-	*Ad hoc* member, Neurological Disorders Program Project Review B Committee (NINDS)
1994-	Council Member of American Society for Neural Transplantation (ASNT), Co-Chairman of Program Committee ASNT
1995-1996	Secretary of ASNT, Chairman of Program Committee, ASNT
1995	Advisory presentation before the US Senate Special Committee on Aging and before the US House Commerce Committee, Health and Environment Subcommittee
1997	Chairman, Advisory committee on Parkinson's disease for presentation to the U.S. Veterans Administration
1998-1999	President of American Society for Neural Transplantation and Repair (ASNTR)
1998-	Member, MDCN-2 Study Section

* * * * *

SELECTED PUBLICATIONS: (161 A. Original articles (99), B. Review articles and chapters (60), C. Books and Editing (2)

A. ORIGINAL ARTICLES

O1. Isacson, O., Brundin, P., Kelly, P.A.T., Gage, F.H. and Björklund, A. (1984) Functional neuronal replacement by grafted striatal neurons in the ibotenic acid lesioned rat striatum. *Nature* 311, 458-460.

O2. Gage, F.H., Dunnett, S.B., Brundin, P., Isacson, O. and Björklund, A. (1984) Intracerebral grafting of embryonic neural cells into the adult host brain: an overview of the cell suspension method and its application. *J. Dev. Neuroscience* 6, 137-151.

O3. Brundin, P., Isacson, O. and Björklund, A. (1985) Monitoring of cell viability in suspensions of embryonic CNS tissue and its use as a criterion for intracerebral graft survival. *Brain Res* 331, 251-259.

O4. Isacson, O., Brundin, P., Gage, F.H. and Björklund, A. (1985) Neural grafting in a rat model of Huntington's disease: Progressive neurochemical changes after neostriatal ibotenate lesions and striatal tissue grafting. *Neuroscience* 16, 799-817.

O5. Gage, F.H., Brundin, P., Isacson, O. and Björklund, A. (1985) Rat fetal brain tissue survive and innervate host brain following five day pregraft tissue storage. *Neuroscience Lett.* 60, 133-137.

O6. Brundin, P., Barbin, G., Isacson, O., Mallat, M., Chamak, B., Prochiantz, A., Gage, F.H. and Björklund, A. (1985) Survival of intracerebrally grafted rat dopamine neurons previously cultured in vitro. *Neuroscience Lett.* 61, 79-84.

O7. Zetterström, T., Brundin, P., Gage, F.H., Sharp, T., Isacson, O., Dunnett, S.B., Ungerstedt, U. and Björklund, A. (1986) In vivo measurement of spontaneous release and metabolism of dopamine from intrastriatal nigral grafts using intracerebral dialysis. *Brain Res* 362, 344-349.

O8. Isacson, O., Dunnett, S.B. and Björklund, A. (1986) Behavioural recovery in an animal model of Huntington's disease. *Proc. Natl. Acad. Sci. USA* 83, 2728-2732.

O9. Brundin, P., Isacson, O., Gage, F.H. and Björklund, A. (1986) Intrastriatal grafting of dopamine-containing neuronal cell suspensions: effects of mixing with target or non-target cells. *Dev. Brain Res.* 24, 77-84.

O10. Brundin, P., Isacson, O., Gage, F.H., Prochiantz, A. and Björklund,A.(1986) The rotating 6-hydroxydopamine lesioned mouse as a model for assessing functional effects of neuronal grafting. *Brain Res.* 366, 346-349.

O11. Sofroniew, M.V., Isacson, O. and Björklund, A. (1986) Cortical grafts prevent atrophy of cholinergic basal nucleus neurons induced by excitotoxic cortical damage. *Brain Res.* 378, 409-415.

O12. Sofroniew, M.V., Pearson, R.C.A., Isacson, O and Björklund, A. (1986) Experimental studies on the induction and prevention of retrograde degeneration of basal forebrain cholinergic neuron. *Prog. Brain Res* 70, 363-389.

O13. Pritzel, M., Isacson, O., Brundin, P., Wiklund, L. and Björklund, A. (1986) Afferent and efferent connections of striatal grafts implanted into the ibotenic acid lesioned neostriatum in adults rats *Exp. Brain Res.* 65, 112-126.

O14. Dunnett, S.B., Whishaw, I.Q., Jones, G.H. and Isacson, O. (1986) Effects of dopamine-rich grafts on conditioned rotation in rats with unilateral 6-hydroxydopamine lesions. *Neurosci. Lett.* 68, 127-133.

O15. Isacson, O., Dawbarn, D., Brundin, P., Gage, F.H., Emson, P.C. and Björklund, A. (1987) Neural grafting in a rat model of Huntington's disease: Striosomal organization as revealed by immunocytochemistry, acetylcholinesterase histochemistry, and receptor autoradiography. *Neuroscience* 22, 481-497.

O16. Isacson, O., Fischer, W., Wictorin, K., Dawbarn, D. and Björklund, A. (1987) Astroglial response in the excitotoxically lesioned neostriatum and its projection areas. *Neuroscience* 20, 1043-1056.

O17. Peschanski M. and Isacson O. (1988) Fetal homotypic transplants in the excitotoxically neuron depleted thalamus I: Light microscopy. *J. Comp. Neurology* 274, 449-463.

O18. Clarke D.J., Dunnett S.B., Isacson O., Sirinathsinghji D.J.S. and Björklund A. (1988) Striatal grafts in rats with unilateral striatal lesions I:Ultrastructural evidence of afferent synaptic inputs from the host nigrostriatal pathway. *Neuroscience* 24, 791-801.

O19. Sirinathsinghji D.J.S, Dunnett S.B., Isacson O., Clarke D.J. and Björklund A. (1988) Striatal grafts in rats with unilateral neostriatal lesions II: *In vivo* monitoring of GABA release in the globus pallidus and substantia nigra. *Neuroscience* 24, 803-811.

O20. Dunnett S.B., Isacson O., Clarke D.J. and Björklund A. (1988) Striatal grafts in rats with unilateral striatal

lesions III: recovery from dopamine dependent motor asymmetry and deficits in skilled paw reaching. *Neuroscience* 24, 813-820.

O21. Brundin P., Barbin G., Strecker R.E., Isacson O., Prochiantz A. and Björklund A. (1988) Survival and function of dissociated rat dopamine neurones grafted at different developmental stages or after being cultured in vitro. *Dev Brain Res* 39, 233-243.

O22. Peschanski M., Rudin M., Isacson O., Delepierre M. and Roques B. (1988) Magnetic resonance imaging of intracerebral neural grafts. *Prog. Brain Res.* 78, 619-625.

O23. Isacson O., Wictorin K., Fischer W., Sofroniew M. and Björklund A. (1988) Fetal cortical suspension grafts to the excitotoxically lesioned neocortex: anatomical and neurochemical studies of trophic interactions. *Prog. Brain Res.*78, 13-27.

O24. Fischer W., Wictorin K., Isacson O. and Björklund A. (1988) Trophic effects on cholinergic striatal interneurones by submaxillary gland transplants. *Prog. Brain Res.* 78, 409-413.

O25. Wictorin, K., Isacson, O., Fischer W., Nothias F., Peschanski M. and Bjorklund A. (1988) Connectivity of striatal gratfts implanted into the ibotenic acid-lesioned striatum I: subcortical afferents. *Neuroscience* 27, 547-562.

O26. Nothias F., Wictorin K., Isacson O., Björklund A. and M. Peschanski (1988) Morpological alteration of thalamic afferents in the excitotoxically lesioned striatum. *Brain Res.* 461, 349-354.

O27. Lams B.E., Isacson O. and Sofroniew M.V. (1988) Loss of transmitter-associated staining following axotomy does not indicate death of brainstem cholinergic neurons. *Brain Res.* 475, 401-406.

O28. Sofroniew, M.V. and Isacson, O. (1988) Distribution of degeneration of cholinergic neurons in the septum following axotomy in different portions of the fimbria fornix: a correlation between the degree of cell loss and the proximity of neuronal somata to the lesion. *J. Chem. Neuroanatomy* 1, 327-337.

O29. Sofroniew M.V., Isacson O. and O'Brien T.S. (1989) Nerve growth factor receptor immunoreactivity in the rat suprachiasmatic nucleus. *Brain Res.* 476, 358-362.

O30. Wictorin, K., Simerly, R.B., Isacson, O., Swanson, L.W. and Bjorklund A. (1989) Connectivity of striatal grafts implanted into ther ibotenic acid lesioned striatum II: efferent projecting graft neurons and their relationship to host afferents within the grafts. *Neuroscience* 30, 313-330.

O31. Isacson O., Riche D., Hantraye Ph, Sofroniew M.V. and Maziere M. (1989) A primate model of Huntington's disease: cross-species implantation of striatal precursor cells to the excitotoxically lesioned baboon caudate-putamen. *Exp. Brain Res.* 75, 213-220.

O32. Dusart, I., Isacson, O., Nothias, F., Gumpel, M. and Peschanski, M. (1989) Schwann cells migrate into CNS excitotoxic lesions. *Neurosci. Lett.* 105, 246-250.

O33. O'Brien T.S., Svendsen C.N., Isacson O. and Sofroniew M. (1990) Loss of true blue labelling from the medial septum following transection of the fimbria-fornix; evidence for the death of cholinergic and non-cholinergic neurons. *Brain Res.* 508:249-56.

O34. Isacson, O., Hantraye P., Maziere M., Sofroniew M.V. and Riche D. (1990) Apomorphine-induced dyskinesias after excitotoxic caudate-putamen lesions and the effects of neural transplantation in non-human primates *Prog. Brain Res.* 82, 523-533.

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B. REVIEW ARTICLES AND BOOK CHAPTERS

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R39. Isacson, O. (1996) Testimony for Hearing before the Subcommittee on Health and Environment of the Committee on Commerce, U.S. House of Representatives: Research Efforts with Respect to Combating Parkinson's Disease and other Neurological Disorders, July 21, 1995, Serial No. 104-68, pp. 52-61.

R40. Isacson, O. and Kordower, J. (1996) The neurobiological and clinical potential of neural cell transplantation: special issue based on the meeting of the American Society for Neural Transplantation, 1995. Cell Transplantation 5, 123-125.

R41. Dinsmore, J.H., Pakzaban, P., Deacon, T.W., Burns, L. and Isacson, O. (1996) Survival of transplanted porcine neural cells treated with F(AB')2 antibody fragments directed against donor MHC class-I in a rodent model. Transplant Proc. 28, 817-818.

R42. Pakzaban, P., Burns, L.H. and Isacson, O. (1996) Xenotransplantation, brain. In: Encyclopedia of Neuroscience 273 (CD Rom).

R43. Isacson, O. (1996) On the Causes and Treatments of Parkinson's Disease. In: Parkinson NPF Report, National Parkinson Foundation, Inc., Vol. XVI1, Issue 1, pp. 8-11.

R44. Galpern, W.R., Tatter, S. and Isacson, O. (1997) Neurotrophic factor protection in models of neurodegeneration: implications for the treatment of neurodegenerative disorders. In: Mitochondria and Free Radicals in Neurodegenerative Diseases, M.F. Beal, N. Howell, I. Bodis-Wollner, eds., John Wiley & Sons, Inc., New York, 557-583.

R45. Isacson, O., Pakzaban, P. and Galpern, W.R. (1997) Transplanting fetal neural xenogeneic cells in Parkinson's and Huntington's disease models. In: Fetal Transplantation in Neurological Diseases, T.B. Freeman and H. Widner, eds., The Humana Press, New Jersey, in press.

R46. Haque, N.S.K., Borghesani, P. and Isacson, O. (1997) Therapeutic strategies for Huntington's disease based on a molecular understanding of the disorder. Molec. Med. Today 3, 175-183.

R47. Schumacher, J.M. and Isacson, O. (1997) Neuronal xenotransplantation in Parkinson's disease. Nat. Med. 3, 474-475.

R48 Isacson, O. and Haque, N. (1997) Gene Therapy of Huntington's Disease. In: Gene Transfer and Therapy for Neurological Disorders, A.E. Chiocca and X. Breakefield, eds., The Humana Press, New Jersey, 423-440.

R49. Isacson, O. and Breakefield, X.O. (1997) Benefits and risks of hosting animal cells in the human brain. Nature Medicine 3, 964-969.

R50. Isacson, O. and Deacon, T. (1997) Neural transplantation studies reveal the brain's capacity for continuous reconstruction. Trends in Neuroscience 20, 477-482.

R51. Sanberg, P., Borlongan, C.V., Wictorin, K., Isacson, O. (1998) Fetal-tissue transplantation for Huntington's disease: Preclinical studies. In: Cell Transplantation for Neurological Disorders, T.B. Freeman and H. Widner, eds., Humana Press, Totowa, New Jersey, 77-93.

R52. Isacson, O., Pakzaban, P., Galpern, W.R. (1998) Transplanting fetal neural xenogeneic cells in Parkinson's and Huntington's disease models. In: Cell Transplantation for Neurological Disorders, T.B. Freeman and H. Widner, eds., Humana Press, Totowa, New Jersey, 189-210.

R53. Isacson, O., Deacon, T. and Schumacher, J. (1998) Immunobiology and Neuroscience of Xenotransplantation in Neurological Disease. In: CNS Regeneration: Basic Science and Clinical Advances, M.H. Tuszynski and J.H. Kordower, eds., Academic Press, San Diego, pp. 365-387.

R54. Emerich, D.F., Kordower, J.H. and Isacson, O. (1998) Cellular Delivery of Neurotrophic Factors as a Potential Treatment for Huntington's Disease. In: CNS Regeneration: Basic Science and Clinical Advances, M.H. Tuszynski and J.H. Kordower, eds., Academic Press, San Diego, pp. 477-504.

R55. Holm, K. and Isacson, O. (1999) Factors intrinsic to the neuron can induce and maintain the ability for neurite outgrowth: a role for bcl-2? Trends Neurosci. 22, 269-273.

R56. Boonman, Z. and Isacson, O. (1999) Caspases in neuronal development and transplantation. Exp. Neurol. 156, 1-15.

R57. Costantini, L.C. and Isacson, O.(1999) Dopamine neuron grafts: development and molecular biology. In: Dopamine Neuron Development, U. di Porzio, R. Pernas-Alonso and C. Perone-Capano, eds., R.G. Landes Company, Georgetown, in press.

R58. Isacson, O., Costantini, L.C. and Galpern, W.R. (1999) Molecules for neuroprotection and regeneration in animal models of Parkinson's disease. In: Innovative animal models of CNS diseases: From molecule to therapy, R. Dean and D.Emerich, eds. Humana Press, Totowa, NJ, in press.

R59. Isacson, O. (1999) The Neurobiology and Neurogenetics of Stem Cells. Brain Pathol., in press.

R60. Isacson, O. and Kang, U.J. (1999) The Potential of Gene Therapy for Treatment of Parkinson's Disease. In: Principles of Surgery for Parkinson's Disease and Movement Disorders, Krauss, K., Jankovic, J., Grossman, R. eds. Lippincott-Raven: Philadelphia, in press.

C. Books and Editing

" Neural grafting in an animal model of Huntington's disease"
author : O. Isacson (Dr Med thesis)
year : 1987
pages : 1-188
ISBN: 91-7900-258-7
distribution: Lund University Library, Box 117 Medical Faculty, S-221 00 Lund, Sweden
or from the author

"Cell Transplantation for Huntington's Disease"
author: P. Sanberg, K. Wictorin and O. Isacson
ISBN: 1-57059-079-6
publisher: R.G. Landes & Co., Austin, TX
(1994)

Total Publications: (161) A. Original articles (99), B. Review articles and chapters (60), C. Books (2)

RESEARCH LECTURES AND INVITATIONS AS SPEAKER:

1. Hamburg (1983) at European Neuroscience Association "Monitoring of neuronal survival in suspensions of embryonic CNS tissue" (paper)
2. Cambridge (1984) at University of Cambridge, Downing Site "Functional neuronal replacement in the ibotenic acid lesioned neostriatum by neostriatal neural grafts" (lecture)
3. Lund (1984) at Nordic Meeting in Neuropsychiatry "Functional neuronal replacement in an animal model of Huntington's disease" (paper)
4. Oxford (1984) at Dept. of Pharmacology, University of Oxford "Striatal neural transplant in the excitotoxically lesioned neostriatum" (lecture)
5. Uppsala (1985) at Nordic Physiology Meeting "Neuronal replacement in an animal model of Huntington's disease (paper)
6. Munchen (1985) at Glial-neuronal communication symposia "The use of neural transplants in the study of lesion models of the adult CNS" (lecture)
7. Oxford (1985) at European Neuroscience Association "Morphological and behavioural changes following neural grafting in rats with lesions of the anteromedial neostriatum" (paper)
8. Avoriaz (1986) Symposium at European Winter Congress on Brain Research "Neural replacement by intracerebral grafts in animal models of Parkinson's and Huntington's disease" (chairman and lecture)
9. New York (1986) at New York Academy of Sciences "Morphology and function of striatal neural grafts" (lecture)
10. Dusseldorf (1986) at Dept. of Neurology "The use of neural grafting in studies of CNS development and

regeneration" (lecture)

11. Spetses-ETP (1986) Research program at European Training Program "Autumn School" "The use of neural grafting in experimental studies of CNS regeneration and development" (lecturer)
12. London (1987) at the Royal Free Hospital, Dept. of Psychiatry "Aspects of degeneration and regeneration in the adult CNS using intracerebral transplants" (lecture)
13. London (1987) at Maudsley Hospital, Inst. of Psychiatry "Neural grafting in animal models of neurodegenerative disease" (lecture)
14. Venice (1987) at the 2nd Symposium on Restorative Neurology "The use of fetal neurons to replace neurons in the CNS" (lecture)
15. Rochester, New York (1987) at Neural transplantation into the mammalian CNS meeting " Fetal cortical grafts into the excitotoxically lesioned neocortex: a model for trophic interactions in Alzheimer's disease ?" (paper)
16. Pécs, Hungary (1987) at Satelite Symposium on Neural Regeneration and Transplantation "Striatal cell suspension grafts in an animal model of Huntington's disease" (paper)
17. Paris (1987) at Dept of Neurology, Fréderic Joliot Hospital, Orsay " A primate model of Huntington's disease"
18. Boston (1988) at Dept. of Neurology, Harvard Medical School, Massachusetts General Hospital "Neuronal Transplantation and strategies for CNS regeneration" (seminar)
19. Paris (1988) at Dept. of Neurology, Frederic Joliot Hospital, Orsay " Excitotoxic lesions models of CNS degeneration" (lecture)
20. Paris (1988) at Dept. of Neurology, Frederic Joliot Hospital, Orsay " The use of neural transplantation in patients with neurodegenerative disease: basic research and recent clinical experiments"
21. Lyon (1988) conference; Trends in Neurobiology "Neuron-target interaction in the CNS: neuronal degeneration and regeneration theories" (paper)
22. Cambridge, England (1989) Neural transplantation meeting: molecular bases to clinical application "Neural transplantation in a primate model of Huntington's disease" (paper)
23. Lund, Sweden (1990) From pharmacological to neuronal replacement in Huntington's disease (paper)
24. Boston, MA (1990) Excitotoxic lesions of the cerebral cortex model degeneration and plasticity seen in neurodegenerative diseases (lecture)
25. Cold Spring Harbor, N.Y. (1990) The use of genetically engineered cells as donor tissue in models of intracerebral transplantation (lecture)
26. Woods Hole Marine Biology Laboratory (1990) RUNN course lecture: Studies of neuronal cell death and regeneration in transplantation models" (faculty)
27. St. Louis, Missouri (1991) CNS Transplants in Adult Damaged Sensory Thalamus and Neocortex (lecture)
28. Washington, D.C. (1991) at Georgetown University, Neural Transplantation in Animal Models of Huntington's Disease (lecture)
29. Paris (1991) at La Salpetriere Hospital, "Animal Models of Neuronal Protection, Degeneration and Regeneration: Concepts of Neuronal Health" (lecture)
30. Stockholm (1991) at Karolinska Institute, "CNS degeneration and regneration models: new concepts of neuronal damage and protection" (lecture)
31. Nagoya, Japan (1992) at "International Conference on Biochemistry of Disease" (lecture)
32. Washington (1992) at "IV International Symposium on Neural Transplantation" (lecture)
33. Brussels (1992) at "25th International Congress of Psychology" (lecture)
34. Frankfurt (1993) Symposium on anti-excitotoxic therapy: *Neuronal protection, gene-transfer and circuitry repair in the basal ganglia* (lecture)
35. Hancock, MA (1994) at Third Berkshire Neuroscience Symposium (lecture)
36. Chatenay-Malabry (Paris) (1994) at 5th International Symposium on Neural Transplantation (lecture)
37. Woods Hole, MA (1994) at RUNN Course "Affecting Neural Function by Transplantation" (faculty)
38. Paris (1995) at ANPP Meeting "Novel Therapeutics in the Nervous System: Gene Transfers and Trophic Factors" (lecture)
39. Chicago, IL (1995) for Rush University Research Week (Keynote speaker)
40. National Press Club, Washington D.C. (1995). New therapies for Parkinson's disease (lecture)

41. U.S. Senate Special Committee on Aging, Washington D.C. (1995). Advisory presentation on Parkinson's disease
42. House Subcommittee on Health and Environment, Washington, D.C. (1995) Advisory presentation on Parkinsons disease
43. Maastricht, Holland (1995) Annual Meeting of NECTAR (lecture)
44. San Francisco, CA (1996) Annual Meeting of American Diabetes Association (Keynote speaker)
45. Miami, FL (1996) University of Miami, "Project to Cure Paralysis" (Visitng Professor)
46. New York, NY (1996) New York Academy of Sciences (lecture)
47. U.S. Veterans Administration, Washington, D.C. (1997) Advisory presentation on Parkinson's disease Chairman, Advisory Committee on Parkinson's disease research
48. Vienna, Austria (1998) Austrian Parkinson Society, Vienna, "Reconnections of neural circuitry in Parkinson's disease patients by xenogeneic dopaminergic neurons." (lecture)
49. New York, NY (1998) 5th Intl. Congress of Parkinson's Disease and Movement Disorders. "Gene Therapy for Parkinsons' Disease", (plenary lecture)
50. Tokyo, Japan (1998) The Molecular Medicine Revolution Conference, "Neural cell transplants to physiologically repair circuitry in neurodegenerative diesease" (lecture).
51. Cardiff, Wales (1998) The Physiological Society, "Cell transplantation as a therapy for Parkinson's disease" (lecture)
52. NewYork, NY (1999) Cornell Medical School/New York Hospital "Developing nerve cells against neurodegeneration" (grand rounds & lecture)
53. Montreux, Switzerland (1999) The Cell Transplant Society, "Primary neuronal cell transplantation for Parkinson's disease (lecture)
54. Lake Tahoe, NV (1999) Keystone Symposia, "Neural xenotransplantation for neurodegenerative disease" (lecture)
55. Halifax, Nova Scotia (1999) Dalhousie University, Clinical Neuroscience (rounds) and Dept. of Anatomy and Neurobiology (lecture)
56. Pittsburgh, PA (1999) University of Pittsburgh Medical Center, Dept. of Pathology (lecture)
57. Rochester, NY (1999) University of Rochester, Experimental Therapeutics Workshop (lecture) and Neurology Grand Rounds
58. Vancouver, BC (1999) XIIIth Intl. Congress on Parkinson's Disease (lecture)
59. Odense, Denmark (1999) 7th Intl. Neural Transplantation Meeting (lecture)
60. Boston, MA (1999) European Behavioral Pharmacology Society and Behavioral Pharmacology Society Conference (lecture)
61. Vienna (1999) Austrian Parkinson Society (lecture)
62. Bonn (1999) Intl. Neuroscience Symposium "Molecular Basis of CNS Disorders" (lecture)
63. London (1999) The Novartis Foundation "Neural Transplantation in Neurodegenerative Disease" (Discussant)