

*Network of European
CNS Transplantation And Restoration*

16th NECTAR meeting



NECTAR

**Sixteenth Annual General Meeting
Amsterdam, 8-10 December 2005**

COLOFON

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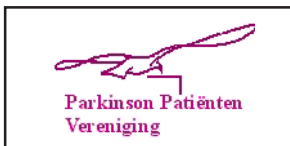
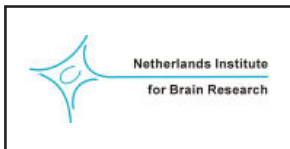
Sponsors

The organizing committee is grateful to the following institutions and companies that supported the meeting financially (in alphabetical order):

Abcam Ltd, Cambridge, UK
Netherlands Institute for Brain Research, Amsterdam, NL
NSGene, Ballerup, Denmark
Parkinson's Disease Society, London, UK
Parkinson Patiënten Vereniging, Bunnik, NL
TEVA UK Ltd, Leeds, UK
The Huntington Disease Association, London, UK

About NECTAR

<http://www.nectar-europe.cf.ac.uk>
<http://www.nesu.mphy.lu.se/nectar>



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PREVIOUS MEETINGS

Munich (D), October 20-21, 1990 (founding meeting)
Le Vesinet (F), May 10-11, 1991 (founding and first annual
general meeting)
Milan (I), January 24-26, 1992
Sandbjerg Manor (DK), November 20-22, 1992
Brussels (B), January 28-29, 1993
Brussels (B), August 27-29, 1993
Amsterdam (NL), October 26-28, 1994
Maastricht (NL), October 26-28, 1995
Amsterdam (NL), October 25-27, 1996
Brussels (B), October 3-5, 1997
Odense (DK), August 25-26, 1998
Amsterdam (NL), December 4-6, 1999
Hannover (D), September 15-17, 2000
Brussels (B), November 30-December 2, 2001
Amsterdam (NL), December 12-14, 2002
Amsterdam (NL), December 4-6, 2003
Brussels (B), December 2-4, 2004
Amsterdam (NL), December 8-10, 2005

The 17th NECTAR meeting will be held in
Freiburg (D), November 30-December 2, 2006

INTRODUCTION

Aim

The overall aim of the meeting is to discuss the progress, current pitfalls and future steps required to reach cellular therapy in human neurodegenerative diseases. Invited speakers of a series of programmed sessions have been asked to survey their experimental or clinical results. In addition, all participants have been invited to communicate their research results with short 8-min (including 2 min discussion) oral presentations in Datablitz sessions.

This year NECTAR commemorates 15 years of active exchange.

Venue

The meeting is held in the Amsterdam Room of NH Grand Hotel Krasnapolsky, Dam 9, 1012 JS Amsterdam, tel +31 20 554 9111, fax +31 20 622 8607, e-mail nhkrasnapolsky@nh-hotels.com, website <http://www.nh-hotels.com>.

The hotel can be reached from Schiphol Airport by Connexion hotel shuttle (one way EUR 11.00, return ticket EUR 17.50), by taxi (EUR 30-40 one way), or by train as it is located at a 7 min walking distance from the Amsterdam Central Railway Station (Amsterdam CS) that has a cheap (EUR 3,40 one way) and rapid 15 min service from and to Schiphol Airport.

Be aware that the Amsterdam taxi system is far from perfect these days often resulting in exorbitant fares.

Registration desk

A registration desk will be open on Thursday, December 8 in the hall in front of the Amsterdam Room of the hotel from 11.00 a.m. to 3.00 p.m., and on Friday, December 9 from 11.00 a.m. to 12.00 p.m. At the desk Tini Eikelboom will be at your service for all information.

For those who have accommodation in the meeting hotel, all room matters should be dealt with at the hotel desk.

On-site registration is possible at the rate of EUR 100 a day.

Arrangement

Your meeting fee of EUR 250 covers attendance, drinks during breaks, and lunches on Friday and Saturday, as well as the meeting dinner on Friday evening.

Meeting dinner

Upon registration you will be asked to sign up for the meeting dinner on Friday evening 8 p.m.. The dinner is included in the meeting package, but *a notice of attendance is obligatory and should be done before noon on Friday December 9*. The dinner will be the famous Dutch Indonesian 'Rijsttafel' and will be served in restaurant Indrapura, Rembrandtplein 42, 1017 CV Amsterdam, tel +31 20 623 7329, e-mail [info@indrapura](mailto:info@indrapura.nl), website www.indrapura.nl (see for location map on page xx).

Instructions for speakers

The 16th NECTAR Meeting has an intense and dense schedule. It is therefore *important that speakers present their talks within the time allotted* (30 min for invited main speakers, and 8 min for Datablitz presentations, both including discussion time). Standard PowerPoint projection facilities are available. During the meeting there will be assistance for your projection.

Publication of abstracts

Abstracts will be published in Cell Transplantation, unless the authors have withdrawn the text for publication in advance or at the meeting.

Thursday afternoon 8 December

13.50 opening by Anne Rosser (chair NECTAR)

Session 1 - Stem cell application on the road to therapy

Chair: Anders Björklund (Lund, Sweden)

14.00 **Elena Cattaneo** (Milan, Italy)

Towards homogeneous clonal neural stem cell lines

14.30 **Paul De Sousa** (Edinburgh, UK)

Isolation of new therapeutically suitable human embryo stem cell lines without direct exposure to animal cell products

15.00 **Thomas Perlmann** (Stockholm, Sweden)

Dopamine neuron determinants in the generation of transplantable dopaminergic cells from mouse embryonic stem cells

15.30 **Jlayi Li** (Lund, Sweden)

A possible cell source of neural transplantation in neurological diseases: critical review on the results with bone marrow stem cells

16.00 break

Session 2 - Datablitzes on stem cells

Chair: Roger Barker (Cambridge, UK)

16.30 **Raimund Hoffrogge** (Rostock, Germany)

2-DE proteomic profiling in neuronal stem cell differentiation

16.38 **Amanda Wintink** (Halifax, Canada)

Dopamine D3 receptor stimulation increases the proliferation of endogenous neural stem cells

16.46 **C. Lange** (Rostock, Germany)

Canonical Wnt signaling during differentiation of the rat striatal progenitor cell line ST14A

16.54 **Eilhard Mix** (Rostock, Germany)

Functional expression of the erythropoietin receptor (EPOR) in the rat striatal progenitor cell line ST14A

17.02 **Vladimir Pekarik** (Cardiff, UK)

Role of FoxP proteins in striatal development

17.10 **Anselme Perrier** (Evry Cedex, France)

Ex vivo/in vivo quality assessment of primate ES derived graft for Huntington disease cell therapy in rodent

17.18 **Jarek Maciaczyk** (Freiburg, Germany)

Transplantation of long-term expanded human fetal neural precursor cells - evidence of distant migration and multi-lineage differentiation

17.26 **Marcel Dihné** (Düsseldorf, Germany)

Purified aggregates of embryonic stem cell-derived immature neurons show reduced teratoma formation after transplantation into the lesioned adult mouse brain

17.34 **Karin Staffin** (Lund, Sweden)

Neural progenitor cell interaction with excitotoxic lesions versus gliomas in the rat striatum

18.00 NECTAR business matters

18.30 dinner break (at your own expense)

PROGRAM

Thursday evening 8 December

Session 3 - Update clinical trials

Chair: Steve Dunnett (Cardiff, UK)

- 21.00 **Ivar Mendez** (Halifax, Canada)
Deep brain stimulation and cell transplantation for
Parkinson's disease: past, present and future
- 21.30 **Christian Spenger** (Stockholm, Sweden)
Olfactory ensheathing glial co-grafts improve functional
recovery in rats with 6-OHDA lesions
- 22.00 **Pierre Krystkowiak** (Lille, France)
Side effects observed in engrafted Huntington's disease
patients

Friday morning 9 December

Session 4 - Regeneration, initiated and stimulated

Chair: Joost Verhaagen (Amsterdam, The Netherlands)

- 09.00 **Hans-Werner Müller** (Düsseldorf, Germany)
Lesion scar and spinal cord repair
- 09.30 **Almudena Ramon Cueto** (Valencia, Spain)
Olfactory ensheathing glia implants to repair spinal cord injuries in rodents and primates
- 10.00 **Stefano Pluchino** (Milan, Italy)
Neural progenitor/stem cells and CNS disorders
- 10.30 **Klaus Leenders** (Groningen, The Netherlands)
The blood brain barrier in Parkinson's disease
- 11.00 break

Session 5 - Neuroprotection revisited

Chair: Patrik Brundin (Lund, Sweden)

- 11.30 **Mark Tuszynski** (La Jolla, CA, USA)
Autologous recombinant nerve growth factor-secreting cells in Alzheimer's disease
- 12.00 **Patrick Aebischer** (Lausanne, Switzerland)
The capsule implant update
- 12.30 **Steven Gill** (Bristol, UK)
Intrapatamenal infusion of glia cell-derived neurotrophic factor to treat Parkinson's disease
- 13.00 lunch break

PROGRAM

Friday afternoon 9 December (1)

Session 6 - Datablitzes on Experimental molecular and cellular therapies

Chair: Guido Nikkhah (Freiburg, Germany)

- 14.00 **Lucy Annett** (Hatfield, UK)
Behavioral responses directed into contralateral compared with ipsilateral space spare dopamine in the striatum following a unilateral 6OHDA lesion of the medial forebrain bundle in rats
- 14.08 **Manolo Carta** (Lund, Sweden)
Role of serotonergic system in the L-DOPA efficacy in the rat model of Parkinson's disease
- 14.16 **Liselijn Wisman** (Lund, Sweden)
Interaction between dopaminergic and cholinergic neurons at the level of the prefrontal cortex on learning and memory
- 14.24 **Ayse Ulusoy** (Lund, Sweden)
Exposure to proteasome inhibitors as a model for Parkinson's disease
- 14.32 **Eduardo Torres** (Cardiff, UK)
Further investigations into young donor age dopamine grafts
- 14.40 **Anna Papazoglou** (Freiburg, Germany)
In vivo characterization of embryonic dopaminergic neurons derived from transgenic mice ectopically expressing Otx2 in the anterior hindbrain
- 14.48 **Harold Robertson** (Halifax, Canada)
Sonic hedgehog: an important factor for neuronal survival for neural transplants in vivo?
- 14.56 **Marco Timmer** (Hannover, Germany)
Development and maintenance of dopaminergic neurons within the substantia nigra in mutant mice of the FGF-2 system
- 15.04 **Vera Baekelandt** (Leuven, Belgium)
Neuroprotective effect of Parkin in the 6-hydroxydopamine rat lesion model for Parkinson's disease
- 15.12 **Yasin Temel** (Maastricht, The Netherlands)
Protection of nigral cell death by bilateral subthalamic nucleus stimulation
- 15.20 **U. Gimsa** (Rostock, Germany)
Gene expression analysis reveals restoration of dopamine metabolism by deep brain stimulation in a rat model
- 15.28 **Claire Kelly** (Cardiff, UK)
A new model to facilitate analysis of xenograft tissue in vivo without the need for immunosuppressant drugs
- 15.36 **Stephan Dunnett** (Cardiff, UK)
Striatal grafts alleviate bilateral striatal lesion deficits in operant delayed alternation in the rat
- 15.44 **Mate Dobrossy** (Cardiff, UK)
Morphological and cellular changes within embryonic striatal grafts associated with enriched environment and involuntary exercise
- 16.00 break

Friday afternoon 9 December (2)**Session 7 - Neuronal supplementation in Parkinson's disease, new aspects**

Chair: Marc Levivier (Brussels, Belgium)

- 16.30 **Alim Louis Benabid** (Grenoble, France)
Long term results of high frequency stimulation of the basal ganglia in severe parkinsonian patients
- 17.00 **Niall Quinn** (London, UK)
On the dyskinesia problem in fetal mesencephalic tissue-engrafted Parkinson's disease patients
- 17.30 **Thomas Carlsson** (Lund, Sweden)
Graft-induced dyskinesia in rats: the role of graft placement and uneven pattern of striatal fiber reinnervation
- 18.00 **Emma Lane** (Lund, Sweden)
The importance of graft size in the development of graft-induced dyskinesia
- 18.30 **Alexander Klein** (Freiburg, Germany)
Functional effects of dopaminergic grafts in the rat model; functional improvements together with impairments
- 20.00 meeting dinner

PROGRAM

Saturday morning 10 December

Session 8 - Datablitzes on Spinal cord system and on Gene transfer

Chair: Patrik Aebischer (Lausanne, Switzerland)

- 09.00 **Angélique Ducray** (Bern, Switzerland)
Differentiation promoting effects of creatine treatment on GABAergic cells in cultures of rat and human spinal cord
- 09.08 **Lev Novikov** (Umeå, Sweden)
Olfactory ensheathing cells promote survival and regeneration of injured rubrospinal neurons
- 09.16 **William Hendriks** (Amsterdam, The Netherlands)
Lentiviral vector-mediated gene transfer of the rat spinal cord lesion scar: failures and potentialities
- 09.24 **Simon Stott** (Lund, Sweden)
Comparison of AAV vector sub-types injected into the neonatal brain
- 09.32 **Bas Blits** (Miami, FL, USA/Amsterdam, The Netherlands)
Neural progenitor cells transduced by a lentiviral vector to express a novel neurotrophin protein implanted into the traumatic injured brain in rats
- 09.40 **Konstantin Cesnulevicius** (Hannover, Germany)
Transfection of primary mesencephalic progenitors: efficient non-viral gene delivery in vitro
- 09.48 **Martijn Tannemaat** (Amsterdam, The Netherlands)
In vivo transduction of the injured rat peripheral nerve by lentiviral vectors as a putative strategy to promote nerve regeneration
- 10.00 break

Session 9 - Gene transfer for therapy

Chair: Gerard Boer (Amsterdam, The Netherlands)

- 10.30 **Mark Tuszynski** (La Jolla, CA, USA)
Adeno-associated viral vector-mediated gene therapy in Alzheimer's disease
- 11.00 **Nicole Deglon** (Orsay, France)
Huntington's disease modeling and treatment: from primary cultures to animal models
- 11.30 **Deniz Kirik** (Lund, Sweden)
Development of new therapeutic strategies for Parkinson's disease based on replacement of L-dopa using rAAV vectors
- 12.00 **Patrik Aebischer** (Lausanne, Switzerland)
Models of Parkinson disease based on lentiviral-mediated overexpression of α -synuclein to help decipher novel therapeutic approaches
- 12.30 closure meeting and lunch

SESSION 1

TOWARDS HOMOGENEOUS CLONAL NEURAL STEM CELL LINES

E. Cattaneo

Department of Pharmacological Sciences and Center of Excellence on Neurodegenerative Diseases, University of Milano, Milano, Italy

Isolation, expansion and neuronal differentiation of neural stem cells *in vivo* and *in vitro* represent important requirements for the biomedical and pharmacological fields. Currently, neural stem and progenitor cells are derived from neural tissue and cultured in aggregates known as neurospheres. However, the proportion of neural stem cells is low and their identity, stability, and physiological relevance uncertain. Furthermore, the cellular complexity of the neurospheres confounds direct experimental interrogation of neural stem cells.

Outside of the brain, a homogeneous source of neural stem cells is represented by pluripotent mouse embryonic stem (ES) cells that expand by symmetrical divisions in adherent monoculture. We have recently optimized novel culture systems for the derivation and the homogenous propagation of adherent neural stem (NS) cells from ES cells via neural lineage commitment of ES cells followed by growth factors addition in basal culture conditions (1). NS cells proliferate continuously in the presence of growth factors, are diploid, clonogenic, homogeneous and long-term stable. After prolonged expansion, they remain able to differentiate efficiently into electrophysiologically active neurons and astrocytes *in vitro* and upon transplantation into the adult brain. NS cells uniformly express morphological and molecular features of neurogenic radial glia, developmental precursors of neurons and astrocytes. Equivalent adherent NS cell lines can readily be established from fetal and adult mouse nervous tissues. Similar NS cells can be generated from human ES cells and human fetal brain.

NS cells represent the first tissue-specific stem cells that can be propagated without accompanying differentiation. As homogenous cultures they can thus be contrasted directly with pluripotent ES cells in the investigation of fundamental properties and biomedical potentials of stem cells. They can also provide a novel system for the pharmacological exploitation and drug screening strategies.

- (1) Conti L.; Pollard S.M.; Gorba T.; Reitano E.; Toselli M.; Biella G.; Sun Y.; Sanzone S.; Ying Q.L.; Cattaneo E.; Smith A. Niche-independent symmetrical self-renewal of a mammalian tissue stem cell. *PLoS Biol.* 3(9):e283; 2005.

Abstract will not be published in Cell Transplantation

ABSTRACTS

ISOLATION OF NEW THERAPEUTICALLY SUITABLE HUMAN EMBRYO STEM CELL LINES WITHOUT DIRECT EXPOSURE TO ANIMAL CELL PRODUCTS

P.A. De Sousa, J.M. Fletcher, P.M. Ferrier, J.O. Gardner, L. Harkness, S. Dhanjal*, J. Harper**, J. Delhanty**, P. Serhal*, R. Mandalam*** and I. Wilmut
Gene Function and Development, Roslin Institute, Edinburgh, UK, *Assisted Conception Unit, University College Hospital, London, UK, **Obstetrics and Gynaecology, University College London, London, UK, ***Geron Corporation, Menlo Park, CA, USA

To realise the therapeutic promise of stem cells and their derivatives, significant challenges must be overcome in culture systems mediating their isolation, expansion and differentiation. First, these systems must produce sufficient quantities of functionally normal cells that are tolerable, or can be made tolerable to the recipient. Secondly, cells produced by these systems must be free of contaminants that could harm the individual, or result in the transmission of disease to the general population. A significant risk factor for the latter is the potential for cross-specific or zoonotic transmission of unknown pathogens that is associated with exposure of cells in culture to animal tissues or undefined tissue products. Recently, we have successfully isolated new human embryo stem cell (hESC) lines on an extracellular matrix substrate of purified human laminin with transitional reliance on mitotically inactivated human fibroblast feeder cells. With this system new hESC lines were isolated using either a defined non-conditioned serum-free medium (NC-SFM) containing only human sourced and recombinant proteins, or media supplemented with a bovine sourced serum replacement product (KNOCKOUT™SR). Both conditions comparably maintained undifferentiated cell markers, a stable karyotype and the potential to form cells representative of all three germinal lineages in vitro and in vivo. The former is thus the first exemplification of a new hESC line derived without direct exposure to any animal cell products, whose potential use in future therapeutic applications would pose a reduced risk of zoonotic pathogen transmission.

SESSION 1

DOPAMINE NEURON DETERMINANTS IN THE GENERATION OF TRANSPLANTABLE DOPAMINERGIC CELLS FROM MOUSE EMBRYONIC STEM CELLS

T. Perlmann^{*/**}, E. Andersson^{**}, U. Marklund^{**}, Q. Deng^{**}, S. Friling^{**}, Z. Alekseenko^{**}, S. Johansson^{**}, J. Jensen^{***}, M. Jönsson^{***}, L. Thompson^{***}, A. Björklund^{***} and J. Ericson^{**}

^{*}Ludwig Institute for Cancer Research; ^{**}Department of Cell and Molecular Biology, Karolinska Institutet, Stockholm, Sweden, ^{***}Wallenberg Neuroscience Center, Lund University, Lund, Sweden

Dopamine (DA) neurons develop from progenitor cells located in the ventral midbrain. We have previously identified the orphan nuclear receptor Nurr1 as a key factor in differentiating DA neurons. However, transcription factors responsible for DA neuron specification operating upstream of Nurr1 have not been identified. The presentation will describe the identification of such transcription factor determinants, termed DPHD1 and DPHD2, functioning in developing proliferating DA progenitor cells. DPHD1 is sufficient and required for the induction of DA neurons while DPHD2 controls the timing of DA cell generation by activating pro-neural bHLH proteins and pan-neuronal characteristics of cell differentiation. Expression of DPHD1 and DPHD2 in embryonic stem cells recapitulates the critical steps in DA neuron differentiation and results in the robust generation of dopamine neurons with a correct midbrain identity. These engineered DA neurons can be transplanted to the striatum of 6-hydroxydopamine lesioned rats where they form extensive tyrosine hydroxylase-positive fiber outgrowths. Together these data show that the elucidation of developmental pathways facilitates the production of authentic DA neurons that ultimately could be used in the treatment of Parkinson's disease.

ABSTRACTS

A POSSIBLE CELL SOURCE OF NEURAL TRANSPLANTATION IN NEUROLOGICAL DISEASES: CRITICAL REVIEW ON THE RESULTS WITH BONE MARROW STEM CELLS

J.-Y. Li

Neuronal Survival Unit, Wallenberg Neuroscience Center,
Department of Experimental Medical Science, Lund University,
Lund, Sweden

Bone marrow contains two major types of stem cells, they are hematopoietic stem cells (HSC) giving rise to all the blood system and mesenchymal (stromal) stem cells (MSC), providing supporting cells and substances. The possibility of generating neural cells from bone marrow-derived stem cells is appealing both conceptually and practically. However, increasing reports indicate that the potential of HSC to generate neural cells is low if any, while it appears that under appropriate experimental conditions MSC has much higher capability to give rise to non-mesenchymal cells, including neural cells. This talk will give a critical review on recent progresses of HSC and MSC in neural differentiation, especially the feasibility, efficacy and controversies. In addition, the possible use of MSC in cell therapy strategies for various neurological diseases will be discussed.

SESSION 2

2-DE PROTEOMIC PROFILING IN NEURONAL STEM CELL DIFFERENTIATION

R. Hoffrogge, S. Beyer and A. Rolfs

Neurobiological Lab, Department of Neurology, University of Rostock, Rostock, Germany

Proteomics has become a powerful tool for the elucidation of neural structures and processes as well as neuronal disease-related protein alterations. In stem cells, the distinctive developmental programs that control self-renewal, differentiation and plasticity can be revealed by proteomic studies. Profiling and functional proteome studies will help to describe the protein inventory as well as protein activity and interactions, sub-cellular localization and post-translational modifications during differentiation processes of stem cells. However, stable neural stem cell lines have not been extensively characterized by proteomic investigations. Based on two dimensional gel electrophoresis (2-DE) combined with mass spectrometric (MS) protein identification, the focus of our work is the detection of changes of protein expression patterns during neural stem cell differentiation. Using the ReNcell VM197 line (ReNeuron, UK), deriving from human ventral mesencephalon, we studied the protein inventory of the stem cells and constructed a 2-DE protein-map with annotations of about 400 spots. The profiling of neuronal differentiation associated changes during the first 7 days of development displayed a large rearrangement of the proteome. We were able to demonstrate significant changes of the expression of 49 identified spots, validated by Western blot analysis for selected proteins, e.g. transgelin-2, PCNA as well as peroxiredoxin 1 and 4. A short-period interval analysis (4 h, 8 h, 16 h, 24 h, 36 h and 72 h) of differentiation related regulations of the protein inventory of the ReNcell VM cells and the stem cell line ST14A, derived from rat striatum, confirms the significant modifications in the proteome profiles during neuronal differentiation. Our approaches demonstrate that the application of proteomic techniques proves to be a valuable tool for the elucidation of the differentiation process of the stem cells to terminally differentiated neurons and offer the possibility for further characterization of specific targets driving the stem cell differentiation. We are just building a proteome data base from neural stem cells.

ABSTRACTS**DOPAMINE D₃ RECEPTOR STIMULATION INCREASES THE PROLIFERATION OF ENDOGENOUS NEURAL STEM CELLS**

A.J. Wintink and H. A. Roberston

The Brain Repair Centre & Laboratory of Molecular Neurobiology, Department of Pharmacology, Dalhousie University, Halifax, NS, Canada

Discovering a pharmacological method of cell replacement therapy for the dopaminergic degeneration characteristic of Parkinson's disease could be useful either alone or in combination with stem cell transplantation therapy. We are currently investigating such a method in rodents using dopamine D₃ receptor stimulation. D₃ receptor stimulation has been shown to induce the proliferation of endogenous neural stem cells that line the subventricular zone (SVZ) using the D2/D3 agonist 7-OH-DPAT. This effect has recently been replicated in our laboratory using two additional and more selective D₃ agonists, one of which was pramipexole. Furthermore, D₃ receptor stimulation selectively increases the proliferation of neural stem cells in the striatum and substantia nigra without a corresponding increase in areas such as the hippocampus. The selectivity of the D₃ effects makes D₃ receptor stimulation a promising target for compensating for the degeneration of cells that are selectively damaged in Parkinson's disease. Interestingly, increasing the proliferation of neural stem cells may be the mechanism underlying the therapeutic efficacy attributed to drugs such as pramipexole in patients with Parkinson's disease. These results support the idea that D₃ receptor stimulation induces the proliferation of neural stem cells and may thus be a viable avenue for cell replacement therapy in Parkinson's disease.

SESSION 2

CANONICAL WNT SIGNALLING DURING DIFFERENTIATION OF THE RAT STRIATAL PROGENITOR CELL LINE ST14A

C. Lange, E. Mix and A. Rolfs

Neurobiological Lab, Dept. of Neurology, Univ. of Rostock, Rostock, Germany

Self-renewal, migration and differentiation of neural progenitor cells are controlled by a variety of pleiotropic signal molecules. Members of the morphogen family of Wnt molecules play a crucial role for developmental and repair mechanisms in the embryonic and adult nervous system. Cell replacement therapy in neurodegenerative diseases requires a detailed understanding of the molecular mechanisms underlying the maintenance and differentiation of neural progenitor cells. Moreover, the use of conditionally immortalized, region-specific cell lines as a model for the differentiation of neural progenitor cells is a promising approach in cell replacement research where pre-differentiation of stem and progenitor cells becomes advantageous for transplantation. A strategy of disclosure of the role of the canonical (β -catenin dependent) Wnt signaling pathway for progenitor cell differentiation is illustrated at the example of the conditionally immortalized rat striatal progenitor cell line ST14A. A shift of culture temperature from permissive 33°C to non-permissive 39°C leads to proliferation stop and start of differentiation into glial and neuronal cells. Investigation of expression of Wnts, Wnt receptors and Wnt-dependent signal pathways on the transcriptional and protein level by RT-PCR, Western blot, immunofluorescence and reporter gene assay point to a stage-dependent involvement of canonical Wnt signaling in the differentiation of ST14A cells, whereas this pathway is suppressed during the proliferation phase. The canonical Wnt molecule Wnt2 is increasingly expressed during differentiation in conjunction with nuclear β -catenin accumulation observed in the late phase of differentiation. The β -catenin dependent transcription is suppressed during proliferation and the early phase of differentiation. The modulation of the Wnt signaling pathways in neural progenitor cells will improve our understanding of differentiation mechanisms of neural progenitor cells *in vitro* and *in vivo* and contribute to the development of standardized procedures for targeted differentiation of neural progenitor cells in order to gain certain cell types for transplantation.

ABSTRACTS

FUNCTIONAL EXPRESSION OF THE ERYTHROPOIETIN RECEPTOR (EPOR) IN THE RAT STRIATAL PROGENITOR CELL LINE ST14A

E. Mix, S. Bogdanow*, T. Büchse*, A. Rolfs and T. Bittorf*
Departments of Neurology and *Medical Biochemistry, Univ. of Rostock, Rostock, Germany

The controlled expansion and differentiation of neural progenitor cells is a challenge of successful cell replacement therapy in neurodegenerative diseases. The regulation of these processes is mediated by several, mainly pleiotropic factors including morphogens and growth factors (3). Erythropoietin (EPO) exerts protective and trophic effects in different regenerating tissues via induction of the JAK/STAT signalling pathway in EPO receptor (EPOR) expressing cells (2). In the model of the well-characterized ST14A (1, 4) progenitor cells that are derived from day 14 rat embryonic striatum, immortalized by retroviral transduction of the temperature-sensitive mutant tsA58/U19 of the SV40 large T antigen and stably transfected with the genes of ciliary neurotrophic factor (CNTF) and glia cell line-derived neurotrophic factor (GDNF), we demonstrate the expression of EPOR on native and transfected ST14A cells by reverse transcription polymerase chain reaction (RT-PCR) and immunoblotting. Furthermore, recombinant human EPO (rhEPO) induced DNA binding of STAT5, a characteristic feature of EPO-dependent signalling, in native and GDNF transfected ST14A cells and of AP-1 in all three cell lines investigated as assessed by radioactive electrophoretic mobility shift assay (EMSA). In native ST14A cells, rhEPO caused a transient increase of proliferation as determined by ³H-thymidine incorporation. The findings are the basis of ongoing investigation of the role of EPO during expansion and differentiation of neural progenitor cells and the manipulation of EPO-dependent signal pathways by small molecules in these cells.

- (1) Böttcher T, Mix E, Koczan D, Bauer P, Pahnke J, Peters S, Weinelt S, Knoblich R, Strauss U, Cattaneo E, Thiesen HJ, Rolfs A. Gene expression profiling of ciliary neurotrophic factor-overexpressing rat striatal progenitor cells (ST14A) indicates improved stress response during the early stage of differentiation. *J. Neurosci. Res.* 73(1):42-53, 2003.
- (2) Körbel S, Büchse T, Prietzsch H, Sasse T, Schumann M, Krause E, Brock J, Bittorf T. Phosphoprotein profiling of erythropoietin receptor-dependent pathways using different proteomic strategies. *Proteomics* 5(1):91-100, 2005.
- (3) Peters S, Mix E, Bauer P, Weinelt S, Schubert B, Knoblich R, Böttcher T, Strauss U, Pahnke J, Cattaneo E, Wree A, Rolfs A. Wnt-5a expression in the rat neuronal progenitor cell line ST14A. *Exp. Brain Res.* 158(2):189-95, 2004.
- (4) Weinelt S, Peters S, Bauer P, Mix E, Haas SJ, Dittmann A, Petrov S, Wree A, Cattaneo E, Knoblich R, Strauss U, Rolfs A. Ciliary neurotrophic factor overexpression in neural progenitor cells (ST14A) increases proliferation, metabolic activity, and resistance to stress during differentiation. *J Neurosci Res.* 71(2):228-36, 2003.

SESSION 2

ROLE OF FOXP PROTEINS IN STRIATAL DEVELOPMENT

V. Pekarik, P. Schoenenberger, T. Oldak and A. Rosser
Brain Repair Group, School of Biosciences, Cardiff University,
Cardiff, Wales, UK

Cell replacement strategies for striatal neurodegeneration based on stem cell differentiation protocols require understanding of genes specifying cellular fate.

In this context, we are interested in FoxP proteins, which belong to a fork-head gene family of transcriptional factors and play an important role in lung and heart development. Their expression profile suggests a role in brain development, especially of the striatum where FoxP1 and 2 are highly up-regulated during the period of peak neurogenesis.

FoxP proteins are transcription repressors whose mode of action and regulation are largely unknown. We have identified that the FoxP function is regulated at the level of the mature protein by changes in the cellular distribution. The protein is localised either in the cell nucleus or in the cytoplasm in a specific pattern in discrete cell population in various brain areas. We have identified a previously unknown ~ 3 kb long 3' un-translated region in mouse FoxP1 mRNA. This region is highly conserved between mouse and human homologs and contains several miRNA binding sites suggesting that it is involved in translational regulation. The target genes of FoxP proteins are virtually unknown, so we have performed a computational prediction of FoxP target genes and are in the process of validating these gene candidates for a role in striatal differentiation. To achieve this we have developed tools to interfere with FoxP signalling (*in vitro* and *in vivo*) including an engineered protein in which the repressor domain has been replaced with viral activation domain.

ABSTRACTS

EX-VIVO/IN VIVO QUALITY ASSESSMENT OF PRIMATE ES DERIVED GRAFT FOR HUNTINGTON'S DISEASE CELL THERAPY IN RODENT

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Huntington's disease (HD) is a devastating monogenic disease affecting 1/10000 adults in Europe. There is no known treatment. The symptoms, which include progressive motor, psychiatric and cognitive dysfunctions, are associated with preferential degeneration of the medium size spiny GABA neurons (MSN) of the striatum. There is presently no way to stop or slow the course of HD. Most of the HD patients die within 15-18 years after the onset of symptoms.

A cell therapy approach via transplantation of striatal fetal tissue has recently shown promising results demonstrating clinical benefits. However, the application of such treatment is restricted because the source of fetal cells is limited, the procurement of human fetal tissue requiring a chain of expertise that cannot be "industrialized". Finding an alternative source of cells suitable to replace efficiently striatal neurons would be a crucial step forward. Human embryonic stem cells (HES) are both pluripotent and capable of unlimited self-renewal. These cells are therefore prime candidates to provide unlimited source of striatal progenitors to be grafted.

Our goal is to validate a protocol of in vitro production of effective neural graft derived from HES cells. Our first objective is to develop a system to easily evaluate the "therapeutic" potential of partially patterned neural population derived from primate ES cells. We are especially interested in evaluating the resistance of this type of graft to the injection procedure and to validate both their proliferative properties and their ability to differentiate into MSN in the specific cellular environment of the cerebral parenchyma. Here we present recent grafting data using an organotypic culture system of neonatal mouse brain (P7). A few thousands of neuronal cells can be observed with this system during up to several weeks after their injection at the level of the striatum in 400 μ m thick brain slices.

SESSION 2

TRANSPLANTATION OF LONG-TERM EXPANDED HUMAN FETAL NEURAL PRECURSOR CELLS – EVIDENCE OF DISTANT MIGRATION AND MULTI-LINEAGE DIFFERENTIATION

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Cell replacement therapy based on human fetal cell transplantation proved to be beneficial under experimental conditions and in clinical trials.

Ethical concerns and limited availability of the aborted tissue make it difficult to become a routine clinical strategy. The purpose of the present work was therefore prolonged in vitro expansion of human fetal-derived neural precursor cells and investigation of their behavior after intracerebral implantation. Human fetal region-specific neural precursors were grafted into intact rodent striata or hippocampi of adult immunosuppressed recipients. All grafts survived up to nine weeks post-transplantation. Numerous intrastrially implanted cells migrated to the adjacent parenchyma and entered white matter tracts to reach distant locations of the host brain such as the forceps minor and the surrounding cortex at the most frontal aspect of the CNS as well as the cerebral peduncle or the substantia nigra.

Migrating cells were characterized by the expression of neuroepithelial stem cell marker nestin and often presented with a particular bi-polar morphology with a long body axis directed along the white matter fiber bundles. Some cells acquired a neuronal morphology with predominant GABAergic differentiation and the expression of marker typical for young migrating neuroblasts (doublecortin). However, most of the implanted cells remained undifferentiated, retaining the nestin expression and typical immature morphology. Intrahippocampal transplants were confined within the target structure and did not enter white matter tracts migrating preferentially towards the dentate gyrus and the hippocampal fissure. There was no difference in phenotypic differentiation between the hippocampal and the intrastriatal grafts. Taken together, these data demonstrates good survival of the human neural xenografts in immunosuppressed animals, extensive migration of a nestin+ cells and relatively limited differentiation of the precursor cells towards more mature neuronal phenotypes such as GABA-producing neurons.

ABSTRACTS**PURIFIED AGGREGATES OF EMBRYONIC STEM CELL-DERIVED IMMATURE NEURONS SHOW REDUCED TERATOMA FORMATION AFTER TRANSPLANTATION INTO THE LESIONED ADULT MOUSE BRAIN**

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Cell therapy is a promising approach to treat neurodegenerative diseases. Embryonic stem (ES) cells can proliferate extensively in an undifferentiated state, thus providing an almost unlimited source of neural precursors after pre-differentiation. However, while teratoma formation rarely occurs in xenogeneic transplantation paradigms, ES cell-derived neural precursor cell populations have been observed to be highly tumorigenic, particularly after allogeneic or syngeneic transplantations, thus posing a major safety problem for these transplantation paradigms. Here we introduce a new differentiation protocol based on the generation of substrate-adherent embryonic stem cell-derived neural aggregates (SENAs) that consist predominantly of neuronally committed precursor cells. Purified SENAs that were differentiated into immature but post-mitotic neurons did not form tumors up to four months after syngeneic transplantation into the acutely degenerated striatum and showed robust survival.

SESSION 2

NEURAL PROGENITOR CELL INTERACTION WITH EXCITOTOXIC LESIONS VERSUS GLIOMAS IN THE RAT STRIATUM

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Neural progenitor cells exhibit a potential for division and migration in response to CNS injury such as stress, epilepsy, stroke and ischemia/hypoxia, with an ability to reconstitute appropriate neurons after neurodegeneration. We wanted to investigate the potential of both endogenous and transplanted NPC cell lines to respond to different types of injury such as excitotoxic lesions or tumor growth. We show that embryonic neural progenitor cell lines transplanted at a distant site from the initial injury or tumor at day 3 are able to migrate towards both types of lesions and co-localize with tumor isles without general spread into the brain parenchyma. Upon encounter with tumor, NPC change phenotype and become vimentin-positive. As shown previously by others we also detected an increase in SVZ neuroblasts stained for *dcx*, as well as a migrative response to excitotoxic lesions in the ipsilateral SVZ as compared with the contralateral unaffected side. However, in case of a growing tumor in the striatum, no significant increase in the *dcx* response was seen in the SVZ. In the presence of transplanted neural progenitor cells, the tumor was approximately 50% smaller compared to control tumors after 1-2 w. Our findings suggest that there exists a population of neural precursor cells which display a tropism for gliomas in the CNS without the use of genetic manipulation. We found that some neural progenitor cells (HiB5, ST14A) in rat have the intrinsic property to target gliomas *in vivo* and to reduce the tumor growth, in some cases (25%) eradicating the tumor completely if co-inoculated into the brain. This antitumor effect elicited by the two progenitor cell lines described have the potential to act on gliomas growing subcutaneously and on a colon carcinoma growing retroperitoneally, which indicate that the phenomenon of tumor inhibition is neither area nor tumor specific.

SESSION 3

DEEP BRAIN STIMULATION AND CELL TRANSPLANTATION FOR PARKINSON'S DISEASE: PAST, PRESENT AND FUTURE

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It has been 20 years since the first human cell transplantation surgery was performed to treat Parkinson's disease. The clinical use of cell transplantation has been built on a strong foundation of basic research in animal models that spans more than 3 decades. In parallel, deep brain stimulation has evolved into a viable neurosurgical option to treat the symptoms of Parkinson's disease. While deep brain stimulation is rapidly becoming a popular surgical strategy for symptom control in Parkinson's disease, the evolution of clinical cell transplantation remains restricted to a few committed centers around the world. The effectiveness and reliability of deep brain stimulation in controlling Parkinson's disease symptoms has made this approach the neurosurgical option of choice when drug therapy fails. However, it is not curative and the disease continues its progressive course. Although, the clinical efficacy of cell transplantation reported in clinical trials to date has been highly variable, the importance of cell transplantation as a strategy for brain repair cannot be dismissed.

This presentation will focus on the nature, advantages and shortcomings of these two therapeutic neurosurgical strategies for Parkinson's disease. Particular emphasis will be given to the neurosurgical techniques used in clinical practice in both approaches. The evidence on clinical efficacy and complications associated with cell transplantation and deep brain stimulation will be reviewed. Finally, the role and the future of cell transplantation in the age of deep brain stimulation will be discussed.

SESSION 3

OLFACTORY ENSHEATHING GLIAL CO-GRAFTS IMPROVE FUNCTIONAL RECOVERY IN RATS WITH 6-OHDA LESIONS

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Parkinson's disease is a progressive neurodegenerative disorder characterized by the degeneration of nigral dopamine neurons. Grafting dopamine neurons from the fetal ventral mesencephalon (VM) to the denervated striatum in animal models and in some patients with severe Parkinson's disease can partly restore functional deficits. However, the transplantation protocols remain experimental because of the limited availability of embryonic tissue, the limited survival of grafts and the restricted dopaminergic reinnervation of host striatum. Olfactory ensheathing cells (OEC) transplanted to the site of a spinal cord injury have been shown to promote axonal sparing / regeneration and functional recovery. Therefore we investigated the effects of OEC to promote axonal outgrowth of grafted dopamine neurons in 6-OHDA lesioned rats.

The results revealed that OEC/VM co-grafts improved amphetamine and apomorphine-induced rotational behaviour compared to VM single-grafts or astrocyte/VM co-grafts. Moreover, only OEC/VM co-grafts gave rise to extensive dopaminergic reinnervation reaching the lateralmost periphery of the striatum. In line with the *in vivo* results, co-cultures of fetal VM and OEC revealed that OEC increased dopamine neurite elongation through a mechanism involving cell-cell contact and releasable factors. We conclude that the addition of OEC enhances the efficacy of fetal dopamine neuron grafts in an animal model of Parkinson's disease.

ABSTRACTS**SIDE EFFECTS OBSERVED IN ENGRAFTED
HUNTINGTON'S DISEASE PATIENTS**

Pierre Krystkowiak

No abstract received

SESSION 4

LESION SCAR AND SPINAL CORD REPAIR

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Traumatic injury of the central nervous system results in formation of a collagenous basement membrane-rich fibrous scar in the lesion center. Due to accumulation of numerous axon growth inhibitory molecules the lesion scar is considered a major impediment for axon regeneration. Following transection of the dorsal corticospinal tract (CST) at thoracic level 8 in adult rats, transient suppression of collagenous scarring in the lesion zone by local application of a potent iron chelator and cyclic adenosine monophosphate resulted in the delay of fibrous scarring. Treated animals displayed long-distance growth of CST axons through the lesion area extending for up to 1.5-2 cm into the distal cord. In addition, the treatment showed a strong neuroprotective effect rescuing cortical motoneurons projecting into the CST that normally die (30%) after thoracic axotomy. Further, anterogradely traced CST axons regenerated through both grey and white matter and developed terminal arborizations in grey matter regions. In contrast to controls, injured animals receiving treatment showed significant functional recovery in the open field, in the horizontal ladder, and CatWalk locomotor tasks (1). We conclude that the fibrous lesion scar plays a pivotal role as a growth barrier for regenerating axons in adult spinal cord and that a delay in fibrotic scarring by local inhibition of collagen biosynthesis and basement membrane deposition is a promising and unique therapeutic strategy to treat human spinal trauma.

Supported by Deutsche Forschungsgemeinschaft (SFB 194, TP B5), Internat. Forschungsinstitut für Paraplegiologie (IFP, Zürich, CH) and Funds of the Research Commission of the Medical Faculty of the Univ. of Düsseldorf

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ABSTRACTS

OLFACTORY ENSEATHING GLIA IMPLANTS TO REPAIR SPINAL CORD INJURIES IN RODENTS AND PRIMATES

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Olfactory ensheathing glia (OEG) transplantation has emerged as a promising repair strategy for spinal cord injuries in mammals. These cells promote functional recovery of rodents with complete spinal cord lesion, and long-distance axonal regeneration in their cords, after transplantation at acute, subacute (1 month) and chronic (4 months) stages. Paraplegic rats recovered voluntary movement and this improvement was not statistically different after grafting acutely or after delayed transplantation. In OEG-transplanted animals, brainstem neuron axons regenerated across the scar and invaded caudal cord stumps up to 3 cm. The extent of axonal regeneration was the same after grafting 1 or 4 months post-lesion, indicating that injured neurons preserve the capacity to respond to growth-promoting strategies at least during four months. The functional outcome of paraplegic animals correlated with the number of regenerated neurons suggesting an implication of them in the recovery of movement. Aiming at translating rodent experience to clinic, we have developed in non-human primates the methodology needed to test the repair efficacy of OEG, using procedures compatible with human practice. OEG can be obtained from the olfactory bulbs of adult primates, causing no-side effects, and thus offering the possibility of autologous transplantation. The cells preserve optimal properties after long-term culture and after freeze and thawing, and hence, one single bulb yields enough OEG for auto- or allo-grafting and storage. We have developed appropriate surgical procedures for bulbectomy and transplantation, behavioral and neurophysiological tests (EMG, TMS, kinematics), and medical treatments for the long-term care of paraplegic monkeys. In conclusion, autologous OEG transplantation is a viable approach in primates, and our results open the prospect for a possible therapy in people with either acute and chronic spinal cord lesions.

Supported by the Ministry of Health, Ministry of Education and Science, Fundación IRSN, and Autonomous Government of Castilla and León.

Abstract will not be published in Cell Transplantation

SESSION 4

NEURAL PROGENITOR/STEM CELLS AND CNS DISORDERS

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We and others have demonstrated that adult neural progenitor cells (aNPC) injected either intravenously (i.v.) or intracerebroventricularly (i.c.) promote multifocal remyelination and functional recovery in mice affected by a chronic-progressive (CP) form of experimental autoimmune encephalomyelitis (EAE), the prototypic animal model for multiple sclerosis (MS) (1). To further explore the role of aNPCs in protecting mice from chronic neurodegeneration during pre-clinical MS, we have transplanted syngenic aNPCs into mice with a relapsing-remitting form of the disease (R-EAE) (2). SJL mice were immunized subcutaneously with proteolipid protein (PLP)139-151 and i.v. injected with 1×10^6 millions of syngenic aNPCs either at the disease onset (13 days post-immunization [dpi]) or after the occurrence of the first clinical relapse (31 dpi). After transplantation, transplanted aNPCs have entered both the brain and the spinal cord, selectively reached inflamed CNS areas, and found to be able to survive for up to 106 days after transplantation. Three months after immunization, mice transplanted at disease onset or after the first relapse have shown a significant ($p < 0.05$) decrease of the disease score, the cumulative score and of the number of relapses. A significant decrease of the extent of demyelination and axonal loss was also found. Interestingly, the majority of the transplanted aNPCs persisting and surviving over time within the CNS around inflamed deep blood vessels maintained an mainly undifferentiated phenotype. Interestingly, transplanted cells accumulated selectively around brain post-capillary venules where glio- and neuro-genic regulators (e.g. BMP-4, Noggin) - possibly contributing to the maintenance of aNPCs in a quiescent state - were focally released. In these perivascular CNS areas, surviving aNPCs induce apoptosis of blood-borne CNS-infiltrating encephalitogenic T cells, thus significantly protecting from chronic neural tissue loss as well as disease-related disability. Thus, undifferentiated aNPCs may have a relevant therapeutic potential in chronic inflammatory CNS disorders as they selectively reach perivenular inflamed area where stem cell regulators are selectively expressed and display in situ remarkable "immune-like" functions promoting long-lasting neuroprotection.

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ABSTRACTS

THE BLOOD-BRAIN BARRIER IN PARKINSON'S DISEASE

K.L. Leenders

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The cause of Parkinson's disease (PD) is unknown. Genetic susceptibility and exposure to neurotoxins are contributors to specific neuronal cell loss in a limited number of PD patients. Association of pesticide exposure with increased risk of PD, and the use of rotenone, paraquat and DDC to create models of selective nigrostriatal degeneration, point to pesticides as candidate neurotoxins.

The link between environment and genetic factors affecting the brain can possibly be found at the level of the blood-brain barrier (BBB) and its protective function. The transport of several drugs across the BBB is largely determined by their physico-chemical properties, with passive diffusion as the major transport process. Direct BBB disruption with leakage has not been found in PD. PGP transporters, part of the ABC family, function as efflux pumps by translocating a substrate to the extracellular compartment. PGP transporter is associated with the multidrug resistance gene (*MDR-1*). At the BBB, PGP is expressed in a high concentration at the luminal side of the brain vascular endothelium. Accumulation of neurotoxic substances by means of decreased PGP efflux function is possibly an important leading event in PD pathology.

Polymorphisms of the *MDR-1* gene, associated with altered PGP expression and efflux pump function at the BBB, may affect the uptake of neurotoxic xenobiotics. Associations between *MDR-1* polymorphisms, PD and pesticides have been found. There is also evidence of protective environmental factors in PD, such as caffeine consumption and cigarette smoking.

Recently, in a pilot study, we found an impaired (on average 18% less compared to age-matched healthy controls) BBB PGP transport system in the midbrain of 5 PD patients by measuring [¹¹C]-verapamil uptake into the brain by PET (1).

These findings in man *in vivo* strongly support the influence of the environmental toxin hypothesis causing PD. After that seminal publication these results are being reinforced by the findings of 20 consecutive PD patients compared to 20 healthy controls.

The primary hypothesis is that in PD specific BBB functions deteriorate with age, possibly on the basis of individual genetic susceptibility, and that the accentuated loss of specific efflux transport capacity (PGP-system) forms a fundamental link between environment (neurotoxin exposure) and genetic predisposition in causing PD.

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SESSION 6

BEHAVIOURAL RESPONSES DIRECTED INTO CONTRALATERAL COMPARED WITH IPSILATERAL SPACE SPARE DOPAMINE IN THE STRIATUM FOLLOWING A UNILATERAL 6OHDA LESION OF THE MEDIAL FOREBRAIN BUNDLE IN RATS

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Forced use of a limb by means of a plaster cast on the opposite limb has been reported to protect dopamine levels in the contralateral striatum following a unilateral 6-hydroxydopamine (6OHDA) lesion (1). Rather than using a plaster cast to force behaviour, the present study used a behavioural task to investigate whether responses directed into contralateral or ipsilateral space following a unilateral 6OHDA lesion influenced the final extent of that lesion. Rats were trained pre-operatively to retrieve coco pops (chocolate flavoured cereal) from five pots placed in front of them in a row. On days 1 to 10 following infusions of 6OHDA into the medial forebrain bundle, rats retrieved a coco pop on 10 trials each day from the pot in far contralateral space (*lesion train contra*; n=7) or far ipsilateral space (*lesion train ipsi*; n=7) or remained in the home cage (*lesion no train*; n=6). Rats with sham infusions of saline also performed the task in far contralateral (*sham train contra*; n=7) or far ipsilateral space (*sham train ipsi*; n=6) or remained in the home cage (*sham no train*; n=6). Amphetamine-induced rotation was less in the *lesion train contra* compared with the *lesion train ipsi* group ($P<0.05$). Similarly, the *lesion train contra* group successfully retrieved more food from the contralateral staircase than the *lesion train ipsi* group ($P<0.05$). On both behavioural measures performance of the *lesion no train* group was intermediate between that of the two other lesion groups. HPLC analysis of dopamine levels measured 4 months after surgery confirmed sparing of dopamine in the striatum in the *lesion train contra* compared with *lesion train ipsi* group (56.8% and 99.1% depletions, respectively, compared with the intact side; $P<0.01$). The results suggest that physical activity may lessen the impact of a dopamine lesion, whereas inactivity may make the lesion worse.

Supported by the Parkinson's Disease Society, UK

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ABSTRACTS

ROLE OF THE SEROTONERGIC SYSTEM IN THE L-DOPA EFFICACY IN THE RAT MODEL OF PARKINSON'S DISEASE

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L-DOPA represents the main pharmacological therapy for the treatment of PD. Despite the fact that it was introduced many decades ago, its mechanism of action is not completely clarified, especially in the last stage of the disease, when the most of the dopaminergic terminals have degenerated.

Since the serotonergic neurons express both the AADC and the VMAT enzymes, it was hypothesized that this system could play a major role in mediating L-DOPA action in the advanced disease.

To test this hypothesis we performed a double lesion experiment as follow:

First, the animals were subjected to an intrastriatal 6OHDA lesion and then treated with daily L-DOPA injections (6 mg/kg i.p.) for three weeks. This treatment is known to induce dyskinesia in about 50% of the animals. The animals were then divided in two groups based on the presence or absence of dyskinesia. Half of the animals in each group were then subjected to a second lesion on the serotonergic system by intraventricular injection of 5,7DHT (150 μ g) which has been shown to deplete 90-95% of the serotonergic innervation in the striatum; the other half of the animals received a sham lesion. After two weeks, rats were again subjected to daily injection of L-DOPA and tested for dyskinesia. The sham lesion group showed a stable expression of dyskinesia, while the double lesioned rats showed a dramatic decrease. None of the previously non-dyskinetic rats developed any signs of dyskinesia. The absence of L-DOPA efficacy in the reversal of the parkinsonian symptoms in the cylinder test suggests that this decrease is due to a diminished conversion rate of L-DOPA to DA in the double lesion group and supports the hypothesis that the serotonergic system is playing a major role in the L-DOPA efficacy and motor complications in the animal model of PD.

SESSION 6

INTERACTION BETWEEN DOPAMINERGIC AND CHOLINERGIC NEURONS AT THE LEVEL OF THE PREFRONTAL CORTEX ON LEARNING AND MEMORY

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Alzheimer's disease (AD) is characterized by a loss of cholinergic neurons, while Parkinson's disease (PD) is characterized by a loss of dopaminergic neurons. Although, these clinical syndromes have originally been described as two distinct diseases, today it is widely accepted that an overlap of clinical and pathological features can be found in these patients. Indeed, published electrophysiological data argues in favor of a convergency of DA and ACh input at the postsynaptic level in the hippocampus. So far, however, no animal model has been developed to study this effect *in vivo*. Therefore, we have lesioned the dopaminergic neurons in the ventral tegmental area (VTA) and the cholinergic neurons in the nucleus basalis magnocellularis (NBM) with 6-OHDA and 192 IgG-saporin, respectively.

Five weeks after surgery, the animals were assessed for deficits in learning and memory in the Morris water maze test. A significant increase in latency to find the platform was found in VTA lesion alone and NBM+VTA lesion groups, but not in NBM lesion alone group. In addition, we also looked at activity levels under basal conditions and after an injection with a low dose of apomorphine. We saw an increase in activity in the VTA lesion alone and the VTA+NBM lesioned animals. No significant difference was found in the skilled paw use test in the staircase between all the groups.

Our findings suggest that the dopamine neurons in the VTA have an important function in learning and memory. However more research is needed to further elucidate whether or not an interaction between dopamine and acetylcholine neurons exist at the level of the prefrontal cortex.

ABSTRACTS

EXPOSURE TO PROTEOSOME INHIBITORS AS A MODEL FOR PARKINSON'S DISEASE

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Current animal models for Parkinson's disease (PD) are based on the reproduction of one of the many pathological processes occurring in PD, therefore, they do not faithfully reproduce the multifactorial progressive degeneration observed in patients. In order to create a better PD model we decided to design a two factor model based on the simultaneous occurrence of two deleterious event in dopaminergic system: first, overexpression of α -synuclein (genetic factor), second systemic PSI injection (environmental factor). For this purpose our first aim was to define a dose of the proteasome inhibitor PSI, causing dysfunction in dopaminergic cells without frank cell loss. PSI has been reported to cause selective dopaminergic degeneration and motor impairments at 18.0 mg/kg dose given over two weeks (1). We performed a dose response study, and tested total doses ranging 4.5-27.0 mg/kg and assessed not only degree of cell loss using stereology but also measured tissue DA content and turnover rates.

Open field locomotor activity tests were performed at 2, 4 and 7 weeks after PSI injection as well as apomorphine and AMPT challenged activity tests. 9.0 mg/kg group showed more pronounced activity changes when they were challenged with apomorphine and AMPT. Nine weeks after the last injection none of the animals showed a deficit in the staircase test, suggesting a minimal or no damage to striatal neurons. While on the other hand, at higher doses we found that PSI could lead to neurodegeneration as 18.0 mg/kg group showed 27% TH+ cell loss in the SN. However, TH enzyme activity as well as DA and HVA levels showed a trend for reduction at 9.0 mg/kg group without a measurable cell loss suggesting that 9.0 mg/kg PSI give sub-threshold impairments.

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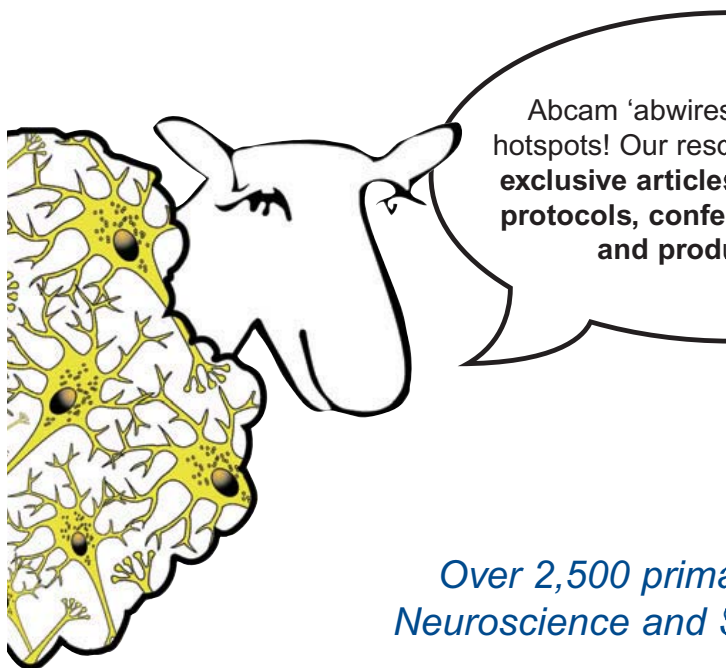
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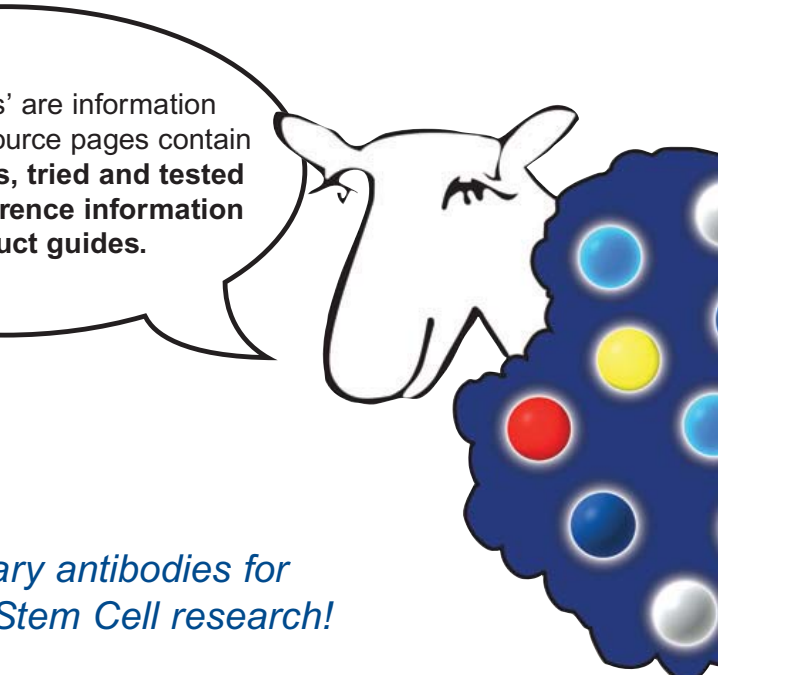


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SESSION 6

FURTHER INVESTIGATIONS INTO YOUNG DONOR AGE DOPAMINE GRAFTS

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The poor survival of dopamine grafts in Parkinson's disease is one of the main factors preventing widespread application of this therapy. We have previously reported high rates of survival of dopamine cells in dopaminergic grafts derived from E11 donor age embryos, much younger than typically investigated. In untreated E11 grafts, dopamine survival was in the range of 20-30% compared to the 5-10% typically reported for E14 derived grafts.

Subsequently we have readdressed the issue of the optimum donor age for dopamine grafts. In a rat model of Parkinson's disease, rats received unilateral 6-OHDA lesions of the median forebrain bundle followed by dopamine grafts derived from embryos of crown rump length 4 mm, 6 mm, 9 mm, or 10.5 mm. Grafts derived from 4mm embryos survived poorly, with less than 1% of the implanted dopamine cells surviving. Grafts derived from 9 mm and 10.5 mm embryos were similar to those seen in previous experiments with survival rates of 8% and 7% respectively. The best survival was seen in the group that received 6mm grafts, which were significantly larger than all other graft groups. Mean cell survival in this group was 36%, an extremely high rate for primary untreated VM cell grafts applied as a single placement and more than five-fold larger than the 10.5 mm group.

As VM tissues of this age contain few differentiated dopamine cells we conclude that the large numbers of dopamine cells seen in the 6mm grafts differentiate and mature post-implantation, and consider the in-vivo conditions which allow this differentiation to take place and the implication of this work for the future of dopamine grafting.

ABSTRACTS

IN VIVO CHARACTERIZATION OF EMBRYONIC DOPAMINERGIC NEURONS DERIVED FROM TRANSGENIC MICE ECTOPICALLY EXPRESSING OTX2 IN THE ANTERIOR HINDBRAIN

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Organizing centres emit signalling molecules that specify different neuronal cell types at precise positions along the anterior-posterior and dorsal-ventral axes of neural tube during development. *Otx2* gene is critical for the specification and regionalization of forebrain and midbrain regions. In transgenic (tg) mice, ectopically *Otx2* expression in the anterior hindbrain using a knock-in strategy into the *En1* locus results in a caudally shift of the midbrain–hindbrain organizer. This leads to an expansion of the midbrain dopaminergic (mid-DA) neuronal population and to a decrease of the hindbrain serotonergic cell group. These changes are preserved in adulthood, and the additional ectopic dopaminergic neurons also project to the striatum.

The purpose of the study is to evaluate the functional restorative properties of the ectopically expanded mid-DA neurons (tgP) *in vivo*. tgP and the respectively wild type (wtP) area as well as ventral mesencephalon (VM) from *Otx2* Tg (tgA) and wild type (wtA) E13 mouse embryos were explanted. Tissue was dissociated and transplanted into the striatum of 6-hydroxydopamine unilaterally lesioned immunosuppressed rats. Lesion and transplantation effects were evaluated by drug-induced rotations. In apomorphine-induced rotations, both VM groups showed a significant compensation 5 weeks after transplantation. Furthermore, we saw a strong tendency of compensation in the tgP group, whereas no such an effect could be seen in wtP. A complete compensation was already observed in amphetamine-induced rotations, not only in the tgA and wtA groups, but also in the tgP group. wtP group showed no signs of recovery. Morphological and stereological analysis showed a significantly higher DA cell survival as well as bigger graft volumes in the wtA group as compared to all other graft groups. tgA and tgP groups showed a significantly higher DA cell survival as compared to the wtP group. In conclusion, ectopically *Otx2* expression in the anterior hindbrain results in caudally expanded mid-DA neurons that demonstrate similar functional properties like their counterparts of the VM.

SESSION 6

SONIC HEDGEHOG: AN IMPORTANT FACTOR FOR NEURONAL SURVIVAL FOR NEURAL TRANSPLANTS IN VIVO?

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Graft survival remains one of the major challenges for neural transplantation.

In theory, the dopamine neurons from a single donor ought to be enough to treat several patients. In practice, 2-7 donors per side have been used, largely because of the very considerable loss (>95%) of dopamine cells. We have been looking for ways of improving graft survival and found by accident that co-culturing E13 ventral mesencephalon cells with neuronal precursor cells led to increased survival of E13 ventral mesencephalon cells in vitro and in vivo (1). In vivo the addition of neuronal precursor cells appears to increase both survival and behavioural effects of a graft by about an order of magnitude. The factor responsible appears to be sonic hedgehog (Shh).

Shh producing neural progenitor cells does more than just increase neuronal survival; they also increase the rate of behavioural recovery. This in turn suggests that Shh affects other processes such as dendrite outgrowth and/or synaptogenesis. In vitro, neuronal precursor cells appear to repulse the neurites of ventral mesencephalon cells. To begin to understand how these processes operate in vivo, we have transplanted ventral mesencephalon tissue adjacent to grafts of neural stem cells. We have also incubated E13 ventral mesencephalon cells with purified recombinant Shh and examined gene expression using DNA microarray technology. Our results suggest that co-grafts of neuronal precursor cells and embryonic dopaminergic neurons might dramatically improve neuronal transplantation for the treatment of Parkinson's disease.

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ABSTRACTS

DEVELOPMENT AND MAINTENANCE OF DOPAMINERGIC NEURONS WITHIN THE SUBSTANTIA NIGRA IN MUTANT MICE OF THE FGF-2 SYSTEM

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Basic fibroblast growth factor (FGF-2) is involved in the development, maintenance, and survival of the nervous system. In the substantia nigra of patients suffering from Parkinson's disease, a profound depletion of FGF-2 in the remaining dopaminergic (DA) neurons was found. In addition, FGF-2 increases dopaminergic graft survival and function in different animal models of Parkinson's disease. FGF receptor (R) signaling is crucial for development and regeneration of the central and peripheral nervous system (1, 2). In the present study, we counted the dopaminergic cells in the substantia nigra pars compacta (SNpc) in FGF-2 knock-out mice, transgenic FGF-2 mice and FGF-R3-deficient mice in comparison to their respective wild-types (wt). Furthermore, we measured the volumes of the SNpc in the different groups (n=6 in each group). The results show a complex picture with regard to the number of tyrosine hydroxylase (TH) positive cells. FGF-R3-deficient mice displayed a reduced number of TH+ cells (6693 +/- 307) as compared to the respective wild-types (8351 +/- 449). FGF-2ko/- mice reveal more DA neurons (7171 +/- 229) within the SNpc than FGF-2ko wt (6057 +/- 284). In addition, transgenic mice (tgFGF-2) have less DA neurons (6902 +/- 414) as compared to the SNpc of the respective wild-types (8032 +/- 414). Currently, we perform a second set of experiments where the role of the endogenous FGF system will be analysed in adult mice after terminal 6-OHDA lesion. The remaining dopaminergic neurons after striatal lesion will be quantified within the SNpc in order to compare the different groups and to examine whether endogenous FGF-2 protects DA neurons from 6-OHDA neurotoxicity.

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SESSION 6

NEUROPROTECTIVE EFFECT OF PARKIN IN THE 6-HYDROXYDOPAMINE RAT LESION MODEL FOR PARKINSON'S DISEASE

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Loss-of-function mutations in the *parkin* gene are the major cause of early-onset familial Parkinson's disease. Parkin is an E3 ligase of the ubiquitin-proteasome pathway involved in protein degradation. Dopaminergic neuron loss may result from the toxic accumulation of parkin substrates, suggesting a key role for parkin in dopaminergic neuron survival. However, the exact mechanism of action of parkin *in vivo* remains to be elucidated. The neurotoxin, 6-hydroxydopamine (6-OHDA) has been widely used to generate rodent models for Parkinson's disease. 6-OHDA induces the generation of reactive oxygen species leading to the degeneration of catecholaminergic neurons, but may also impair proteasome activity. In this study, we have investigated the neuroprotective capacity of parkin in the 6-OHDA rat model. Lentiviral vector-mediated overexpression of parkin in the substantia nigra prior to a striatal 6-OHDA lesion led to a significant preservation of dopaminergic cell bodies and nerve terminals. Moreover, lesioned rats overexpressing parkin displayed a corresponding behavioural improvement. Our results demonstrate that parkin acts as a potent neuroprotective agent *in vivo* and support its therapeutic potential for the treatment of not only familial but also sporadic Parkinson's disease.

ABSTRACTS

PROTECTION OF NIGRAL CELL DEATH BY BILATERAL SUBTHALAMIC NUCLEUS STIMULATION

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In Parkinson's disease (PD), the subthalamic nucleus (STN) becomes hyperactive (disinhibited) which causes excitotoxic damage to midbrain dopaminergic neurons. Here, we examined whether silencing of the hyperactive STN by chronic bilateral deep brain stimulation (DBS) increased the survival of midbrain dopaminergic neurons in the bilateral 6-OHDA rat model of PD. Three groups of rats were used in this study. Rats in group A underwent sham surgery, rats in group B received bilateral injections of 6-OHDA into the striatum, and rats in group C received both bilateral 6-OHDA injections into the striatum and bilateral electrodes in the STN. DBS with clinically relevant parameters started one week after the 6-OHDA injections and lasted for 3 months. After 3 months all rats were sacrificed. High-precision design-based stereologic examination of the total number of nigral dopaminergic neurons (Nissl staining) and tyrosine hydroxylase (TH) immunoreactive neurons revealed that STN DBS resulted in a significant survival of these neurons. These data provide the first evidence in vivo that bilateral STN DBS is useful for protecting midbrain dopaminergic neurons from cell death in an experimental model of PD.

This study was supported by grants from the Dutch Medical Research Council (ZonMw), no: 940-37-027 and the Dutch Brain Foundation (Hersenstichting Nederland) nos 10F02.13, 10F03.19 and 10F04.17

SESSION 6

GENE EXPRESSION ANALYSIS REVEALS RESTORATION OF DOPAMINE METABOLISM BY DEEP BRAIN STIMULATION IN A RAT MODEL

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High-frequency stimulation (HFS) of nucleus subthalamicus (STN) is a therapeutic option for late-stage Parkinson's disease. The actual mechanism of action is not fully understood yet. We performed gene expression analyses in a rat model of Parkinson's disease, i.e. striatal 6-OHDA lesion. Using Affymetrix microarrays, we compared gene expression in healthy and lesioned male Wistar rats with implanted electrodes with and without stimulation for 3 hours. As a control, we analyzed gene expression in rats which had not been manipulated. We found the specific regulation of genes belonging to groups involved in neuroprotection, inflammation, transcription, structural functions, cell metabolism, extracellular matrix, and signal transduction. Additionally, a significant down-regulation of tyrosine hydroxylase (TH) mRNA expression induced by lesion and a TH upregulation by HFS in lesioned rats could be observed. It is highly plausible that HFS has an excitatory influence on the stimulated structure itself which may not necessarily result in enhanced function of down-stream neural structures.

ABSTRACTS

A NEW ANIMAL MODEL TO FACILITATE ANALYSIS OF XENOGRAFT TISSUE *IN VIVO* WITHOUT THE NEED FOR IMMUNOSUPPRESSANT DRUGS

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Xenotransplantation of human neural tissue into the host rodent brain is important for preclinical studies of cell replacement therapy. However, the requirement for immunosuppression of the host to prevent graft rejection severely restricts the host survival times and thus limits the opportunity for the cells to properly differentiate and reduces the information that can be obtained. An animal model system is required that would allow long term analysis of these grafts *in vivo*. We describe a novel animal model in which rats are tolerised in the neonatal period, and subsequently do not require immunosuppression following intracerebral implantation of human cells as adults.

Animals that received an ip injection of primary or 10 day expanded human tissue in the neonatal period subsequently supported intrastriatal grafts of the same tissue in the adult without immunosuppression. Animals that received an intrastriatal neonatal graft followed by an adult intrastriatal graft in the contralateral striatum did not support the graft. Where primary tissue was used, different foetal donors were used for the ip tolerisation and adult transplant.

In contrast to the commonly used administration of immunosuppressant drugs such as Cyclosporin A, this model system is cheaper and results in long term survival of intracerebral xenografts, thus allowing more detailed analysis of the graft.

SESSION 6

STRIATAL GRAFTS ALLEVIATE BILATERAL STRIATAL LESION DEFICITS IN OPERANT DELAYED ALTERNATION IN THE RAT

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In order to assess the capacity of striatal grafts to alleviate cognitive deficits of the frontal type that arise following bilateral striatal lesions, control, lesion and grafted rats were tested in an operant test of delayed alternation (1). Bilateral striatal lesions induced a marked impairment in choice accuracy, and signal detection analysis indicated that the lesion animals were reliably impaired on both parametric and non-parametric indices of discriminative sensitivity but not of response bias. The impairment was apparent at all intertrial interval delays, including the very shortest, suggesting the deficit involves frontal-type executive function, rather than is one of short term memory. The grafted animals exhibited a significant alleviation of the deficit, again apparent at all delays. Histological analyses indicated good graft survival, and injections of a dextran amine anterograde tracer bilaterally into the host prefrontal cortex indicated reformation of extensive projections into the grafted tissues. Since performance of the operant delayed alternation task is dependent upon the integrity of corticostriatal connections (1), which is disrupted bilaterally by the lesions and restored in the transplanted animals, the results corroborate the hypothesis that striatal grafts can alleviate complex cognitive functions of the frontal type by a mechanism that involves functional integration of the grafted neurons into the neural circuits of the host brain.

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ABSTRACTS

MORPHOLOGICAL AND CELLULAR CHANGES WITHIN EMBRYONIC STRIATAL GRAFTS ASSOCIATED WITH ENRICHED ENVIRONMENT AND INVOLUNTARY EXERCISE

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Environmental enrichment and exercise are factors that have been implicated in influencing behaviour and altering neuronal processes associated with cellular morphology in both “normal” and injured states of the central nervous system. Using a rodent model of Huntington’s disease, we investigated whether chronic environmental complexity and involuntary exercise can affect morphological and cellular changes within embryonic striatal transplants placed into the experimentally lesioned adult striatum. Adult female rats were trained on the bilateral version of the Staircase test - requiring fine motor control to reach and collect reward pellets - prior to being lesioned unilaterally in the dorso-striatum with Quinolinic acid. The lesioned animals received E15 whole ganglionic eminence cell suspension grafts followed by the exposure to either enriched environment, or to a standard housing with or without daily treadmill exercise. The grafted animals showed significant functional recovery on both the Staircase test and the drug-induced rotation. Neither the housing conditions nor the training had an impact on the behaviour with the exception of the treadmill reducing the ipsilateral drug-induced rotation observed amongst the lesioned animals. However, the animals housed in the enriched environment had significantly increased striatal brain-derived neurotrophic factor (BDNF) levels, and the grafted cells in these animals had both greater spine densities and had larger cell volumes. The animals exposed to the treadmill exercise scheme had both reduced BDNF levels and grafted cells showed decreased spine densities. The study suggests that the environment and the experience of the animal can affect the plasticity of transplanted cells. Appropriately exploiting the underlying, and yet unknown, mechanisms could lead the way to improved anatomical, and potentially functional, integration of the graft.

SESSION 7

LONG TERM RESULTS OF HIGH FREQUENCY STIMULATION OF THE BASAL GANGLIA IN SEVERE PARKINSONIAN PATIENTS

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Surgery was the main treatment for Parkinson's disease (PD) in the pre-L-dopa era and became later the main alternative when medication fails. The discovery of lesion-like effects of high frequency stimulation (HFS) opened a new era for surgical treatment due to the reversibility, adaptability and low morbidity of the method. Initiated in the thalamic Vim nucleus for the control of tremor, HFS became applied to another target of ablative methods, the internal Pallidum GPi, and thereafter to a new theoretical target, the subthalamic nucleus (STN). The latter appeared to be an extremely efficient target on the triad "tremor, rigidity and bradykinesia" and applied to several thousands of patients in the last decade. The criteria for successful surgery are correct patient selection and careful and precise placement of the electrode. The best predictor of the efficiency of HFS of STN, is the preoperative response to L-dopa. The short-term benefits of bilateral stimulation of STN have been well documented, but the long-term outcomes of the procedure are unknown.

In a 5-year prospective study, the first 49 consecutive patients were assessed at 1, 3 and 5 y with and without L-dopa, using the UPDRS. As compared with base line, the patients' scores at 5 y for motor function while off-medication improved by 54% ($P<0.001$) and those for activities of daily living by 49% ($P<0.001$). Speech was the only motor function for which off-medication scores did not improve. The scores for motor function on-medication did not improve at 1 y, except for the dyskinesia scores. On-medication akinesia, speech, postural stability and freezing of gait worsened between 1 and 5 y ($P<0.001$). At 5 years, the dose of dopaminergic treatment and the duration and severity of L-dopa-induced dyskinesia were reduced as compared with base line ($P<0.001$). The average scores for cognitive performance remained unchanged, but dementia developed in three patients after 3 y. Mean depression scores remained unchanged. Severe adverse events included a large intracerebral hemorrhage in one patient. One patient committed suicide.

Patients with advanced PD treated with bilateral STN stimulation had marked improvements over 5 y in motor function while off-medication and in dyskinesia while on medication. There was no control group, but worsening of akinesia, speech, postural stability, freezing of gait and cognitive function between 1-5 y is consistent with the natural history of PD. However, individual follow-up shows that patients can be separated into three groups: 25% regularly worsen, consistently with the natural history of PD, 36% stable within a 10% range of the UPDRS III score and 38% improve from an average 56 to 40 on the UPDRS scale within 1 y and half of them sustain a continuous improvement over 5 y. This is consistent with experimental data in rodents and monkeys supporting the hypothesis of a neuroprotective effect of STN stimulation. This warrants further controlled clinical trials with potential impact on the management of PD patients. The mechanisms of action are not fully understood and might associate inhibition of cell firing, jamming of neuronal message and exhaustion of neurotransmitter release at the synaptic level. The future of HFS depends on technical improvements, on the exploration of new targets, as well as on the improvement of alternative methods currently being developed and explored.

ABSTRACTS

ON THE DYSKINESIA PROBLEM IN FETAL MESENCEPHALIC TISSUE-ENGRAFTED PARKINSON'S DISEASE PATIENTS

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“Off-treatment” mobile dyskinesias (OTDs) are very rare in ungrafted, but common, and sometimes disabling, in grafted PD patients. It has been suggested that OTDs result from excessive dopamine release from grafts or, because of their semiological resemblance to diphasic dyskinesias, to sub-optimal levels of dopaminergic stimulation.

In patients grafted in Lund : OTD severity does not correlate with the degree of clinical improvement, with the maximum F-dopa uptake on PET, or with the magnitude of its increase after grafting (1), or with basal or methamphetamine-induced dopamine release (2). Their semiology resembles those in the Tampa (3) [but not the Denver; (4)] series, primarily involving the leg, with elements of stereotypy, ballism and mobile choreo-dystonia reminiscent of diphasic dyskinesias. However, they were unchanged after a supra-threshold dose of L-dopa, arguing against an analogous mechanism.

The mechanism of OTDs may relate to non-dopaminergic effects of the graft, or possibly to focal imbalance between dopamine release and striatal dopamine receptor hypersensitivity.

In two London patients successfully withdrawn from L-dopa for two or more years who developed OTDs, typical “on-drug” dyskinesias were absent, yet consistently recurred for 1-2 hours after 6-monthly L-dopa challenges, suggesting that “priming” to L-dopa may be irreversible.

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SESSION 7

GRAFT-INDUCED DYSKINESIA IN RATS: THE ROLE OF GRAFT PLACEMENT AND UNEVEN PATTERN OF STRIATAL FIBER REINNERVATION

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Transplantation fetal ventral mesencephalic (VM) cells into the striatum of patients with advanced Parkinson's disease (PD) have been used as a therapeutical intervention since the early 90s. However in two recent double-blind clinical trials, investigating the efficacy of striatal transplants, a significant proportion of the patients receiving grafts developed dyskinetic side effects. These new type of graft-induced dyskinesia were not seen in the patients receiving sham operations. In one of the trials a follow-up study investigated the ^{18}F -DOPA uptake in the striatum and compared the dyskinetic and non-dyskinetic grafted patients to each other. This study showed that the grafted patients that developed graft-induced dyskinesia exhibited an uneven pattern of striatal reinnervation in the ventral-lateral sector of the putamen in comparison to the grafted non-dyskinetic patients. In order of these findings we designed a study to investigate the role of graft placement in the induction of dyskinesias as well as the effect on already established L-DOPA-induced dyskinesia in parkinsonian rats. Briefly, abnormal involuntary movements, resembling peak-dose dyskinesias seen in PD patients, were induced in complete 6-OHDA lesioned rats by daily injections of L-DOPA for six weeks. The selected moderate-to-severe dyskinetic animals received single grafts of about 130,000 fetal ventral mesencephalic cells, either in the rostral or in the caudal aspect of the head of striatum. The animals were then followed up for 30 weeks, during which time they were frequently tested for dyskinetic behaviors. Our results show that rats grafted in the caudal part of the head of striatum induce graft-induced dyskinesias when the transplanted cells were activated by amphetamine. The severity of these abnormal involuntary movements was significantly correlated with a difference in the graft-derived Tyrosine hydroxylase-positive fiber reinnervation in the caudal aspect of the head of striatum relative to the rostral part. Furthermore, the same caudal grafts were the most effective to reduce the already established L-DOPA-induced limb and orolingual dyskinesia, which is predominantly seen as hyperkinetic behavior. The following data suggests that the graft-induced dyskinesias in PD patients may be linked to single, small graft deposits that provide an uneven, patchy reinnervation in the putamen.

ABSTRACTS

THE IMPORTANCE OF GRAFT SIZE IN THE DEVELOPMENT OF GRAFT-INDUCED DYSKINESIA

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Intra-striatal transplantation of embryonic ventral mesencephalon can restore striatal dopamine and improve motor symptoms in Parkinson's disease patients. Following transplantation, a sub-population of patients experience a significant worsening of 'off' medication dyskinesia. This study examined whether the size of the transplant could influence the incidence and severity of dyskinetic behaviours in the 6-hydroxydopamine (6-OHDA) lesioned rat. Female Sprague-Dawley rats with 6-OHDA lesions of the nigrostriatal dopamine pathway were treated for 21 days with L-dopa (12 mg/kg) plus benserazide (15 mg/kg). Vehicle, or tissue suspension containing the equivalent of 3/4 (large) or 1/8 (small) of an embryonic (E14) ventral mesencephalon was stereotaxically injected into the lateral striatum, yielding 280 ± 63 and 17408 ± 1229 TH positive cells in the small and large grafts, respectively. Motor function and L-dopa-induced abnormal involuntary movements (AIMs) were evaluated prior to transplantation and at regular intervals post-grafting. Both groups of grafted animals demonstrated a progressive improvement in forelimb use asymmetry and amphetamine-induced ipsilateral rotations. L-dopa induced AIMs were however reduced only in the group with large transplants. A few animals with large grafts demonstrated mild abnormal movements 12-24 h post L-dopa at 2 and 8 weeks after transplantation. These movements consisted of spontaneous contralateral turns, axial twisting and forepaw tapping. All the rats with large grafts exhibited robust AIMs of the mouth, limb and body axis after amphetamine administration, which were coincident with the production of contralateral rotations. Using the selective dopamine or serotonin uptake inhibitors, GRB 12909 and fluvoxamine respectively, we determined that these behaviours are mediated through dopamine dependent mechanisms. However, limb and orolingual movements were significantly more severe after simultaneous blockade of dopamine and serotonin reuptake, suggesting that serotonergic mechanisms may contribute to graft-induced dyskinesia. Our results indicate that plastic striatal changes responsible for dyskinesia persist post-transplantation.

SESSION 7

FUNCTIONAL EFFECTS OF DOPAMINERGIC GRAFTS IN THE RAT MODEL; FUNCTIONAL IMPROVEMENTS TOGETHER WITH IMPAIRMENTS

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Unilateral injections of 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle (MFB) of rats lead to a degeneration of dopaminergic (DAergic) neurons and to a permanent impairment of skilled limb movements, especially in forelimb reaching tasks and gait performance. To substitute the loss of cells and their functions cell replacement therapies were established in the past but -despite some evidence for recovery in animal models and clinical trials- they failed to restore the basal ganglia circuitry completely. The mechanisms and qualitative aspects of graft-induced recovery are still largely unclear.

We investigated whether the transplantation of fetal DAergic neurons mediates recovery of motor pattern by rewiring neuronal pathways or supports compensatory mechanisms to counteract motor deficits. The rats were trained in skilled reaching tasks (single pellet grasping task for qualitative and quantitative evaluation, staircase test for quantitative evaluation). Gait analysis (rung walking task and footprint analysis) was performed to test skilled walking and forelimb/hindlimb coordination. Rotational behaviour was assessed after unilateral 6-OHDA MFB lesion and intrastriatal transplantation of E14 ventral mesencephalon (VM)-derived cell suspension.

After the transplantation grafted rats showed overcompensation under amphetamine administration and a reduction of apomorphine-induced rotation. This indicates significant graft survival and graft effects on simple motor behaviour.

Morphological and stereological analyses also demonstrated substantial graft survival and reinnervation. After transplantation an incomplete but significant improvement of gait performance could be observed for the test parameters "*distance between feet*", "*limb rotation*" and "*stride length*". The paw reaching test demonstrated significant functional improvements: more pellets eaten by the transplanted (+62.5%) than by the sham-transplanted animals. By contrast, single pellet grasping task revealed that some components of a rats' grasping movement (such as *supination of paw* and *release of pellet*) benefited from the graft; some were not affected at all, and, very interestingly, some became even more impaired (predominantly at the beginning and initiation of a grasping movement such as e.g. "*orient*", "*limb lift*" and "*aim*"). However, mean performance rate failed to show any graft-induced effects.

In conclusion, functional recovery in skilled forelimb performance in rats after ectopic transplantation of VM-derived cells might be due to graft-induced compensatory mechanisms. A full restoration of skilled forelimb motor pattern might indeed require a more complete and organotypic reconstruction of the mesotelencephalic DAergic pathway.

Supported by Deutsche Forschungsgemeinschaft Ni-330, Graduate School Freiburg and the Alberta Heritage Foundation for Medical Research

ABSTRACTS

DIFFERENTIATION PROMOTING EFFECTS OF CREATINE TREATMENT ON GABAERGIC CELLS IN CULTURES OF RAT AND HUMAN SPINAL CORD

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The creatine/phosphocreatine system plays an important role in the maintenance of cellular energy homeostasis in cells with high and fluctuating energy demands like neurons. We have previously shown that creatine treatment promoted the GABAergic phenotype in striatal cultures. In the present study, we aimed at investigating the effects of creatine (5 mM) supplementation on survival and differentiation of cultured spinal cord neurons. For that purpose dissociated rat (E14) and human (first trimester) spinal cord cultures were prepared and grown for one week. First, we demonstrated that both isoforms of creatine kinase are expressed in rat and human fetal tissues. Chronic creatine treatment for 7 days (DIV7) resulted in a significant increase of GABA-immunoreactive (-ir) cell densities both in rat and human cultures (by 25% and 40%, respectively), while the total neuronal cell number and general viability, were not affected. Similar results were obtained for short term (DIV5-7) creatine exposure in rat cultures, suggesting a differentiation inducing effect of creatine. Creatine administration, however, failed to affect morphological parameters of GABAergic neurons, i.e. soma size, neurite length and number of branching points/neuron. Preliminary data show that creatine supplementation exerts partial neuroprotection against 3-nitropropionic acid induced toxicity. In sum, the data show that creatine exposure promotes differentiation and has potential neuroprotective effects on cultured GABAergic spinal cord neurons. Our findings suggest that creatine plays an important role in cell fate decision during development of spinal cord neurons.

SESSION 8

OLFACTORY ENSHEATHING CELLS PROMOTE SURVIVAL AND REGENERATION OF INJURED RUBROSPINAL NEURONS

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Olfactory ensheathing cells (OECs) have been shown to support axonal regeneration and functional recovery after spinal cord injury (1, 4) and their growth-promoting effect could be attributed to the expression and secretion of neurotrophic factors. We have previously demonstrated that neurotrophins can rescue neurons of long spinal tracts from retrograde cell death and reduce posttraumatic cavitation (2, 3). The present study investigates the effects of OEC on survival, collateral sprouting and axonal regeneration of rubrospinal neurons after cervical spinal cord injury in adult rats.

OECs were purified using a method of differential adhesion and cultured for 3 or 7 weeks with forskolin and rhGGF2. About 95% of OECs in culture were immunopositive for low affinity NGF receptors p75 and S-100 protein. After cervical C4 hemisection, OECs were transplanted into lateral funiculus at 1 mm rostral and caudal from the lesion site. Neuronal survival and regeneration were assessed using retrograde labeling with fluorescent tracer Fast Blue, anterograde tracing with biotinylated dextran amine and immunostaining for neuronal markers.

Cervical hemisection induced 50% loss of rubrospinal neurons at 8 weeks postoperatively. The density of rubrospinal axons in the dorsolateral funiculus was reduced to 30% of normal control and no regeneration was found in the trauma zone. Transplantation of OECs cultured for 3 weeks rescued significant proportion of rubrospinal neurons from retrograde cell death, increased the density of rubrospinal fibers in the dorsolateral funiculus and gray matter rostral to the lesion and promoted regeneration of rubrospinal axons across the injury site. In contrast, OECs cultured for 7 weeks had no positive effects on neuronal survival or axonal regeneration.

The results indicate that OECs promote neuronal survival and regeneration after spinal cord injury and that culture conditions could affect OECs efficacy to stimulate spinal cord repair.

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ABSTRACTS

LENTIVIRAL VECTOR-MEDIATED GENE TRANSFER TO THE NEURAL SCAR AFTER RAT SPINAL CORD INJURY: FAILURES AND POTENTIALITIES

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Viral vector-mediated overexpression of e.g. neurotrophins in cells constituting the neural scar may represent a powerful approach to render neural scar tissue of a CNS lesion permissive for neuronal regrowth. In this study a lentiviral vector encoding green fluorescent protein (LV-GFP) was injected in and around the scar two weeks after a dorsal column lesion in the rat spinal cord in order to characterize cell types transduced in the neural scar. GFP expression was found at all time points after injection, and increased from 4 to 7 days with no apparent difference between 7 and 14 days. The core of the lesion was virtually devoid of GFP expression despite direct vector injections in this area. Based on colocalisation of GFP with specific cell markers (GFAP, vimentin, Raldh2, NeuN, OX-42, ED-1 and NG-2), predominantly astrocytes in the rim of the lesion were transduced, whereas neurons, microglia, oligodendrocyte precursors and macrophages were transduced to a lesser extent. No Raldh2-positive meningeal cells, present in the core of the scar, expressed GFP. *In vitro* meningeal cells were readily transduced, indicating that *in vivo* the formation of an extracellular matrix might prevent LV particles to transduce cells in the core of the scar. Since astrocytes are important cellular constituents of the glial scar after CNS injury transduction of astrocytes with LV vectors encoding neurotrophic factors like BDNF or NT-3 may lead to enhanced regeneration of severed axonal tracts through or along boundaries of a CNS lesion.

SESSION 8

COMPARISON OF AAV VECTOR SUB-TYPES INJECTED INTO THE NEONATAL BRAIN

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Recombinant viral vector delivery is a powerful tool in disease modeling of CNS neurodegenerative conditions. Similarly, viral vectors that mediate over expression or down regulation of a particular gene involved in CNS development provide a valuable method to study, e.g. axon guidance/target innervation. In the present study, we compared the efficacy of the recombinant AAV (rAAV) vectors carrying stereotype AAV 1, 2 or 5 capsids packaging AAV2 genome (denoted below as rAAV 2/1, rAAV 2/2, and rAAV 2/5). All vectors were engineered to encode the green fluorescent protein (GFP). Each vector was delivered to the striatum or the lateral ventricle of neonatal Sprague Dawley rat pups (0-2 days after birth). Four weeks after surgery, the animals were killed for histological analysis, where the distribution of GFP positive cells was assessed in the brain. We found that there was a thorough and robust transduction in the forebrain of animals injected with the rAAV2/5 vector. Unilateral delivery to the striatum resulted in an 80% transduction of striatal projection neurons on the injected side. Widespread expression of GFP was also observed in the cortex on the injected side. In contrast, very few GFP positive cells were found on the un-injected side. We are currently investigating whether the rAAV 2/1 or rAAV 2/2 vectors can provide a similar distribution of expression.

ABSTRACTS**NEURAL PROGENITOR CELLS TRANSDUCED BY A LENTIVIRAL VECTOR TO EXPRESS A NOVEL NEUROTROPHIN PROTEIN IMPLANTED INTO THE TRAUMATIC INJURED BRAIN IN RATS**

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This study investigates transplanted neural progenitor cells (NPCs) as a potential reparative strategy for the injured brain following traumatic brain injury (TBI), specifically the efficacy of NPCs transduced to secrete a novel recombinant protein, multilineurotrophin (MNT) designed to functionally bind to multiple epitopes of the neurotrophin family of receptors (trkA, trkB and trkC). These modified NPCs may serve as a delivery vehicle for the sustained release of bioactive neurotrophic factors to provide neuroprotection after injury or reverse the histopathological and behavioral changes evident following TBI. Male Sprague-Dawley rats underwent moderate fluid-percussion injury. NPCs obtained from E13.5 fetal rat neocortical tissue were transduced to express the MNT gene utilizing a lentiviral vector. One week post-injury, the NSCs genetically modified to secrete MNT were stereotactically implanted near the injury epicenter ventral to the internal capsule targeting the ipsilateral cortex, hippocampal CA3 region, and myelinating oligodendroglia underlying the white matter tracts. Transplanted NPCs are found to survive 8-12 weeks following transplantation into the injured brain and are located mainly at the injury core as well as localized along the white matter tracts underlying the cortical layer. TBI animals transplanted with NPCs expressing the MNT gene have reduced contusion volumes and better preserved cortical cytoarchitecture as compared to animals with TBI transplanted with cells lacking this gene. Behavioral testing (grid walk task, sensorimotor placing test and water maze) in rats transplanted with NSCs has been performed to measure the possible ameliorative effects of NSC transplants on TBI-induced sensorimotor and cognitive deficits. These findings suggest that the utilization of cellular transplantation strategies exert a beneficial effect on outcome following TBI.

Supported by NS30291

SESSION 8

TRANSFECTION OF PRIMARY MESENCEPHALIC PROGENITORS: EFFICIENT NON-VIRAL GENE DELIVERY *IN VITRO*

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Neuronal progenitor cells play an important role in potential regenerative therapeutic strategies in different neurodegenerative diseases, like Parkinson's disease. However, survival of transplanted cells is still limited and the identification of grafted cells *in situ* remains difficult. It was recently shown that neuronal progenitors provide attractive perspectives in neurodegenerative diseases and could be even more promising with regard to a better survival when transfected with (a) neurotrophic factor(s). Therefore, we investigated the possibilities to transfect mesencephalic neuronal progenitors with different constructs including expression reporters EGFP and DsRed, and constructs carrying neurotrophic factor genes. We used different techniques for transfection: lipofection with Lipofectamine 2000 reagent (Invitrogen), electroporation with EasyJecT Optima electroporator (EquiBio Ltd), and nucleofection with Nucleofector device (Amaxa GmbH). Ventral mesencephalic progenitors were gathered from rat embryos (E12) (1) and expanded in culture for 3 days prior to transfection. The highest transfection rate of up to 47% was achieved using nucleofection. Viability of the transfected cells 6 hours after transfection was about 40%. Furthermore, mesencephalic neuronal progenitors survived the transfection procedure and differentiated into TH+ neurons. Within the group of transfected cells, many progenitors (nestin+; GFAP-), including several neurons (β III-tubulin+) were found. In order to provide the progenitor cells with their own trophic factor, the 18 kDa isoform of FGF-2 was introduced. Functional tests, like cell viability assay (WST-1) and cell proliferation assay (BrdU-ELISA) were performed to follow the behaviour of the transfected cells *in vitro*. This study shows, that neural progenitors provide attractive perspectives in neurodegenerative diseases and could be even more promising when transfected with neurotrophic factor.

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ABSTRACTS

IN VIVO TRANSDUCTION OF THE INJURED RAT PERIPHERAL NERVE BY LENTIVIRAL VECTORS AS A PUTATIVE STRATEGY TO PROMOTE NERVE REGENERATION

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Peripheral nerve regeneration following transection and repair in patients is limited and functional impairments always remain. This has motivated us to search for new clinically applicable strategies to improve outcome of nerve repair. Exogenously applied neurotrophic factors such as nerve growth factor (NGF) and glial cell-derived neurotrophic factor (GDNF) have the potential of enhancing regeneration. To exert a beneficial effect, however, they must be applied locally, preferably distal to the lesion site and, most likely, for a prolonged period of time. These conditions can be met through viral vector-mediated gene transfer, i.e., transduction of neural cells to produce neurotrophic factors locally.

Therefore, we investigated whether in vivo transduction of Schwann cells by direct injection of lentiviral (LV) vectors coding for NGF and GDNF leads to an improvement of outcome after transection and immediate surgical reconnection of the rat sciatic nerve. Lentiviral vectors integrate their gene into the target cell DNA and have been shown previously to give non-toxic long-term transgene expression in Schwann cells. The left sciatic nerve was transected and directly reconstituted with microsutures in 47 female Wistar rats. Immediately after repair, LV-NGF (n=13), LV-GDNF (n=14) or LV-“stealth”-GFP as a control vector (n=14) was microinjected in the distal nerve stump as a 3 µl volume containing 6×10^7 viral vector particles, as evaluated with p24 ELISA titering. No vector was injected in 8 animals serving as a reference group.

Animals were tested weekly for return of sensory function to the left hindpaw with the footflick test and return of motor function by measuring the sciatic function index (SFI) and with the “Catwalk” automated gait analysis. From the NGF, GDNF and sGFP groups six animals were sacrificed at 4 weeks. All other animals were sacrificed at 14 weeks. Histological analysis included quantification of nerve fiber outgrowth on transverse sections 10 mm distal to the lesion site using a neurofilament antibody to visualize all nerve fibres, a CGRP antibody to stain nociceptive sensory nerve fibres and a ChAt antibody to stain motoneuron fibres. *In situ* hybridisation for NGF and BDNF of longitudinal sections of the transection/repair site was performed to assess transgene expression at 4 and 14 weeks.

The tentative results of this experiment will be presented.

SESSION 9

ADENO-ASSOCIATED VIRAL VECTOR-MEDIATED GENE THERAPY IN ALZHEIMER'S DISEASE

Mark Tuszynski

No abstract received

ABSTRACTS

HUNTINGTON'S DISEASE MODELING AND TREATMENT: FROM PRIMARY CULTURES TO ANIMAL MODELS

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The identification of disease-causing genes in familial forms of neurodegenerative disorders and the development of genetic models closely replicating human CNS pathologies have drastically changed our understanding of the molecular events leading to neuronal cell death. If these achievements open new opportunities of therapeutic interventions, including gene-based therapies, efficient delivery systems taking into account the specificity of the central nervous system are required to administer therapeutic candidates. In addition, there is a need to develop genetic models in large animals that replicate late stages of the diseases for pre-clinical studies.

Over the last few years, we have investigated the potential use of lentiviral vectors as tool to model and treat Huntington's disease. We have shown that mutant htt overexpression in the striatum of adult rats results in a selective and severe neuropathology characterized by a characterized by the sequential appearance of ubiquitinated htt aggregates, neuronal dysfunction and cell death. Recently, we have scaled-up the approach in non-human primates and assessed the behavioral deficits associated with the striatal pathology. A selective increase in locomotor activity, average speed under apomorphine stimulation and abnormal movements (choreic-like, dystonic abnormal movements) were observed in animals injected with mutant htt. In parallel, we have investigated the neuroprotective effect of various molecules, including the ciliary neurotrophic factor. Recent progresses toward lentiviral-mediated gene transfer of therapeutic candidates will be presented.

SESSION 9

DEVELOPMENT OF NEW THERAPEUTIC STRATEGIES FOR PARKINSON'S DISEASE BASED ON REPLACEMENT OF L-DOPA USING RAAV VECTORS

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PD is a progressive neurological disease characterized by extensive loss of the DA producing neurons of the substantia nigra (SN). Typically, symptoms of PD start to appear when about 70-80% of striatal dopamine is lost and about 50% of the dopamine neurons in the SN have degenerated. Oral administration of L-3,4-dihydroxyphenylalanine (L-DOPA) is currently the most effective and widely used medical treatment for PD. Despite the fact that L-DOPA remains efficacious in reversing the motor symptoms of the disease, as the disease progresses, patients receiving this treatment suffer from multiple side effects, most importantly wearing-off, on-off swings, and disabling dyskinesias. Clinical observations suggest that continuous infusion of L-DOPA (or long-acting DA agonists) may alleviate the dyskinetic side effects. Indeed, there is considerable evidence that side effects can be greatly reduced if L-DOPA is administered at a constant level, and that continuous L-DOPA delivery may provide a more physiological stimulation of denervated striatal DA receptors.

Recombinant AAV vectors are currently being developed to deliver L-DOPA continuously in the brain as a means to restore the depleted dopamine in the striatum. With the development of new generation, high-titer rAAV vectors, it has become possible to express TH stably and at sufficiently high levels in the striatum and when combined with co-expression of the primary rate-limiting synthetic enzyme for BH₄ synthesis, GTP-cyclohydrolase 1 (GTPCH1), it was possible to demonstrate sufficient L-DOPA production in animals with nigral DA neuron lesions. We showed recently that centrally produced L-DOPA is functional and reverses the Parkinsonian symptoms in animals. Furthermore, we showed that dyskinesias induced by systemic pulsatile L-DOPA delivery can effectively be reversed by *in vivo* gene transfer using the same vectors. These results demonstrate that L-DOPA delivery by intrastriatal delivery of rAAV vectors can be developed into a novel therapy for PD.

ABSTRACTS

MODELS OF PARKINSON DISEASE BASED ON LENTIVIRAL-MEDIATED OVEREXPRESSION OF α -SYNUCLEIN TO HELP DECIPHER NOVEL THERAPEUTIC APPROACHES

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During the last decade, at least six genes have been linked to familial Parkinson disease (PD). It is believed that the molecular understanding of the consequences of these gene mutations leading to nigral dopaminergic degeneration will help to devise new therapies for PD. A large set of data suggests that α -synuclein may be a central molecule not only for rare familial forms of the disease, but also for sporadic cases making it a key player for the understanding of the degeneration mechanism and for the development of novel therapies. Of great importance is the development of relevant genetic models of PD that can be used to screen various therapeutic approaches. The development of transgenic mice based on the overexpression of mutated α -synuclein has been rather disappointing as none of the transgenic mice are associated with death of the nigral dopaminergic neurons. Our laboratory is studying the ability of lentiviral vectors either injected directly in the substantia nigra or in the perivitelin space to create genetic rat models of PD. Recently, we have reported that the direct nigral injections of lentiviral vectors expressing mutated α -synuclein induces the development of aggregates in nigral dopaminergic neurons leading to death within 6 weeks of injection. Using this genetic model, we have recently observed that lentiviral-mediated overexpression of parkin or heat shock proteins prevent the α -synuclein-induced dopaminergic degeneration, opening new avenues for a gene therapy treatment of PD. We believe that this rat α -synuclein model also constitutes a promising model to test small drugs knowing that α -synuclein is likely to play an important role in sporadic PD. Various molecules including heat shock protein inducers, stimulators of autophagy and small molecules derived from a drosophila high throughput screen are being presently tested.

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RESTAURANT ROUTES

Several routes brings you to Restaurant Indrapura at the Rembrandtsplein for the Dutch-Indonesian 'Rijsttafel' on Friday evening.

1

Nieuwezijds Voorburgwal route brings you along the Amsterdam Historical Museum, the famous Spui (former Amsterdam Provo Center of the 60ties), the Aula and Library of the University of Amsterdam, the Flower Market and the Gay center of the city. The longest route that takes a walk of 25 min maximally.

2

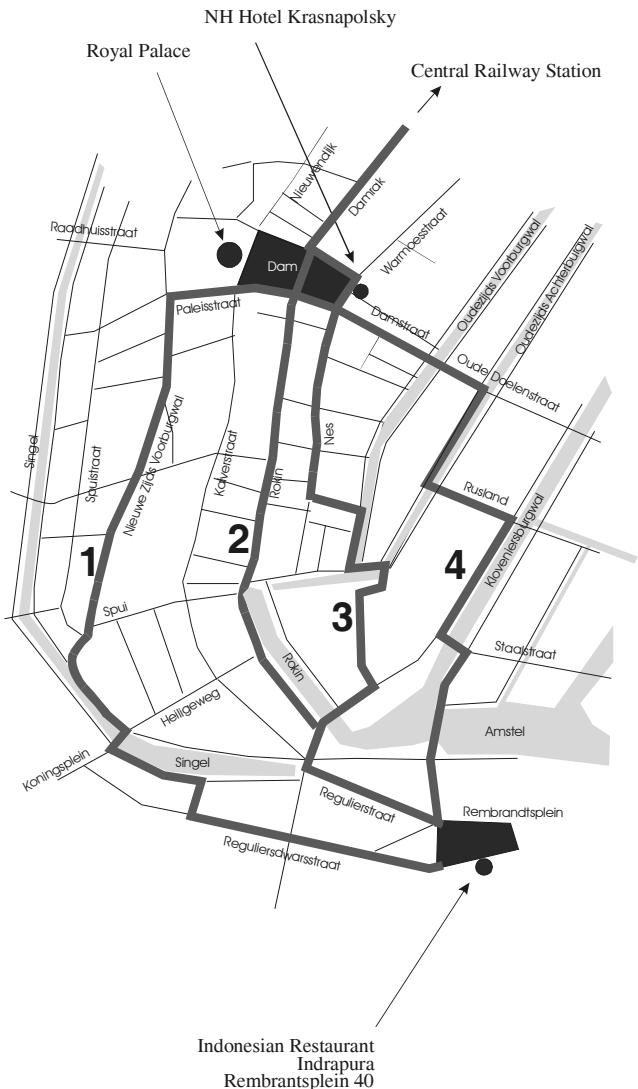
Rokin route is the most direct route to the Rembrandtsplein and shows you the construction works of the new North-South track of the Amsterdam Metro and many shops on crowded streets.

3

Nes route is to be seen as the historical 'maze' route through the oldest parts of Amsterdam with many 16th century houses and passing through the University of Amsterdam buildings. Find your route through the small streets!

4

Rusland route brings you to the border of the Red Light District to enjoy the smell of weed and thereafter via the former main canal between the Amstel river and the 17th century port of Amsterdam (Kloveniersburgwal) to the Amstel where you have a nice view on the Opera house before you enter the Rembrandtsplein.



Tot ziens

