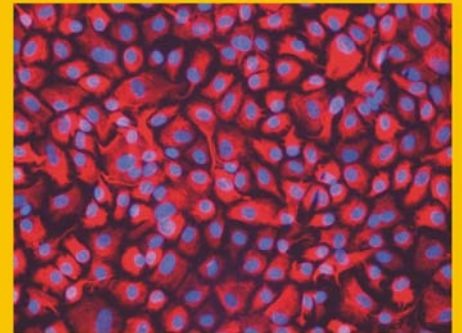


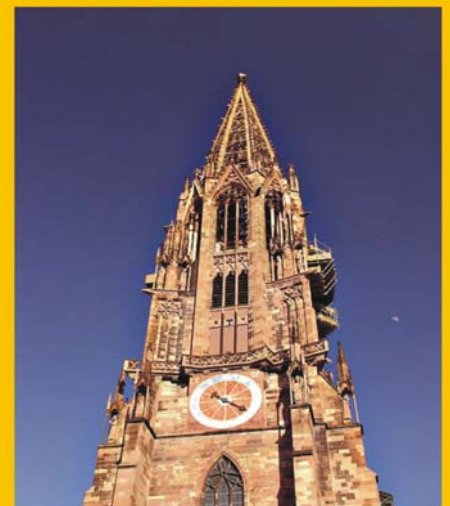
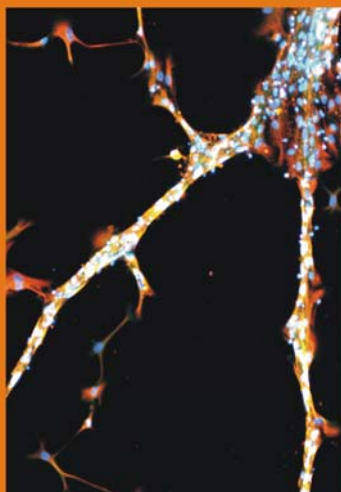
17th NECTAR MEETING

Network of European CNS
Transplantation and Restoration



30.Nov.-02.Dec.2006

Freiburg/Germany



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INTRODUCTION

INTRODUCTION

Dear Colleagues,

It is a great pleasure for us to welcome you to the 17th NECTAR Annual General Meeting from November 30th to December 2nd in Freiburg.

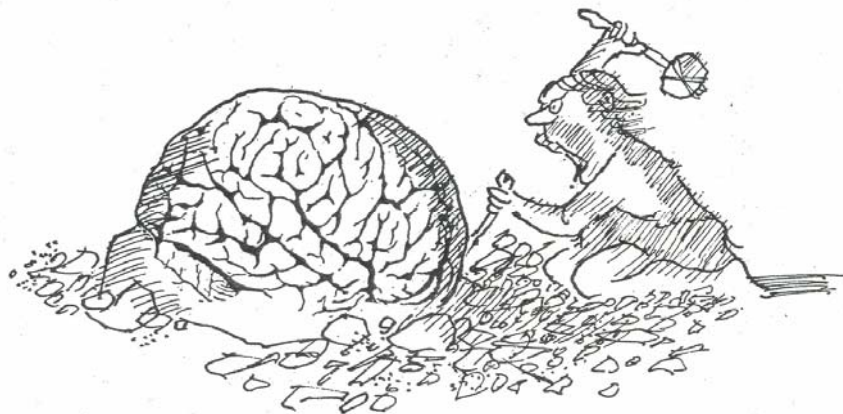
The present programme will continue in our "NECTAR tradition" focussing on experimental studies and clinical applications of cellular and restorative therapies for the treatment of neurodegenerative diseases.

The scientific programme committee and members of the NECTAR board have composed a programme that highlights the current progress of basic and clinical science in the highly dynamic field of neurorestoration and its intensive interdisciplinary exchange with related areas, such as gene therapy, neuro-imaging and developmental neurobiology. Leading scientists and clinicians will address key issues in this translational field of research accompanied by many young scientists that will present their ongoing research in focussed datablitz presentations. We sincerely hope that this concept continues to stimulate an intensive exchange of personal and professional expertise and thereby fosters NECTAR's original goals to serve as an excellent communication platform and European network.

We are looking forward to welcoming you cordially in Freiburg!

Guido Nikkhah

(On behalf of the scientific and local organising committees)



Congress secretary

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Anne Rosser	Gregor Wenning
Christian Winkler	Gerard J. Boer
Roger Barker	Angela Cenci

Local Organizing committee

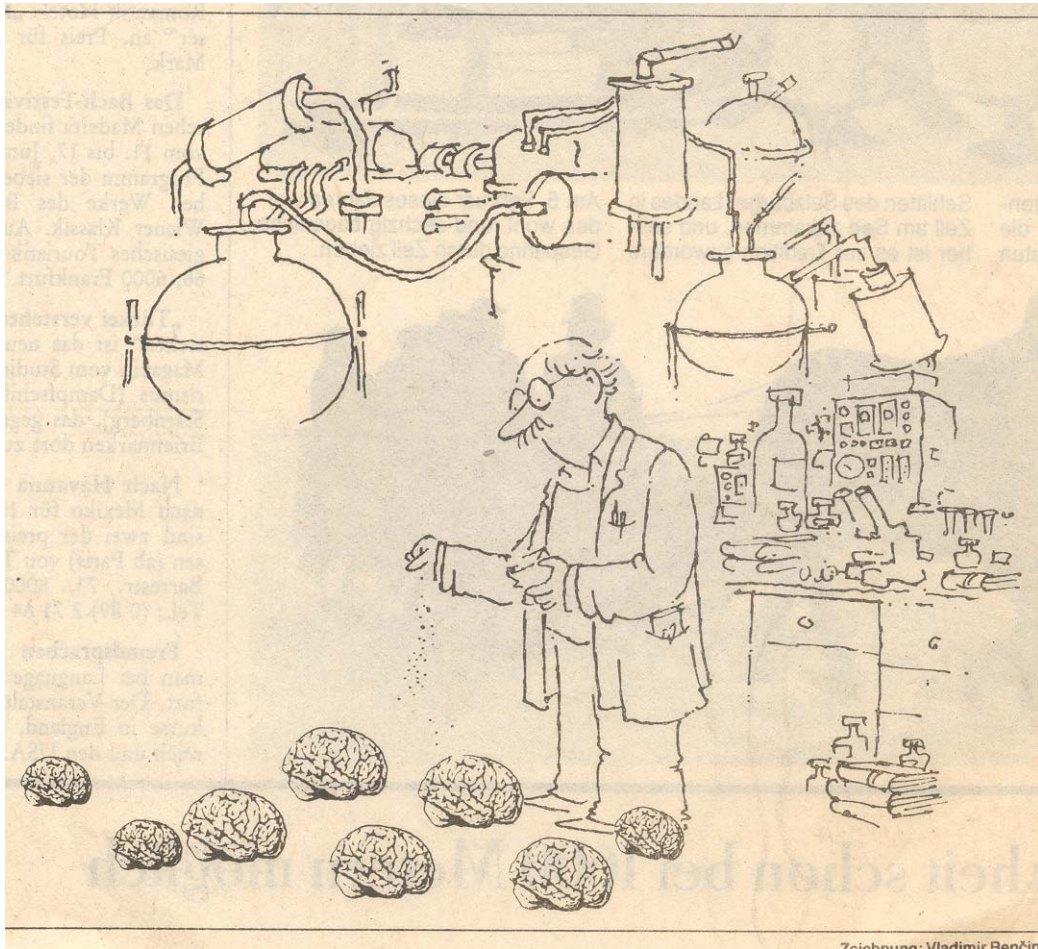
Guido Nikkhah	Manuela Fellmann (secretary)
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Previous NECTAR Meetings

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

SCIENTIFIC PROGRAMME



12:00h-19:00h	On-site registration
14:00h	Opening by Guido Nikkhah and Deniz Kirik (President NECTAR)
14:00h-16:00h	Session I <u>Parkinson's Disease - Clinical</u> Chair: Stephen Dunnett (Cardiff, UK)
<i>14:15h-14:40h</i>	Per Odin (Bremerhaven, Germany) <i>Pharmacotherapy in Parkinson's Disease</i>
<i>14:40h-15:05h</i>	Andreas Kupsch (Berlin, Germany) <i>Deep Brain Stimulation in Parkinson's Disease: Criteria for Patient Selection</i>
<i>15:05h-15:30h</i>	Roger Barker (Cambridge, UK) <i>The future of cell therapies in the treatment of Parkinson's disease</i>
15:30h-16:00h	Datablitz Session I <u>Stem Cells and Parkinson's Disease</u> Chair: Roger Barker (Cambridge, UK)
<i>15:30h-15:38h</i>	Anna-Maria Szczesniak (Halifax, Canada) <i>Co-grafting as a method for improving survival of transplanted ventral mesencephalon neurons</i>
<i>15:38h-15:46h</i>	Martin Krause (Freiburg, Germany) <i>Green fluorescent protein (GFP) transgenic rats: A new tool for transplantation</i>
<i>15:46h-15:52h</i>	Lachlan Thompson (Lund, Sweden) <i>Intra-nigral grafting of foetal ventral mesencephalon from the TH-GFP mouse reveals graft-derived dopaminergic innervation of the host forebrain</i>
<i>15:52h-16:00h</i>	Volker Tronnier (Lübeck, Germany) <i>In vitro differentiation of neural stem cells isolated from the adult brain</i>
16:00h-16:30h	Coffee Break

Thursday

30.Nov.06

16:30h-19:30h

16:30h-19:00h

Session II

Huntington's Disease - Clinical

Chair: Marc Peschanski (Creteil, France)

16:30h-17:00h

Stephen Dunnett (Cardiff, UK) and
Roger Barker (Cambridge, UK)

UK transplant trial of fetal striatal tissue in mild-moderate Huntington's disease – An Update

17:00h-17:30h

Marc Peschanski (Creteil, France)

Effect of fetal neural transplants in patients with Huntington's disease 6 years after surgery: a long-term follow-up study

17:30h-18:00h

Guido Nikkhah (Freiburg, Germany)

Transplantation of fetal striatal cells in patients with Huntington's disease (HD): the German experience within a European-based multi-center study

18:00h-18:30h

Pasquale Gallina (Florence, Italy)

*Human fetal transplantation in Huntington's disease
First Italian clinical trial at the University of Florence:
Preliminary report*

18:30h-19:00h

Datablitz Session II

Huntington's Disease

Chair: Marc Peschanski

18:30h-18:38h

Philipp Capetian (Freiburg, Germany)

*Histological evaluation of six months post transplantation striatal fetal stem cell grafts in a Huntington's disease patient:
Signs of ongoing mitosis, neurogenesis, beginning maturation and integration*

18:38h-18:46h

Gunnar Hargus (Hamburg, Germany)

The extracellular matrix molecule tenascin-R enhances neuronal differentiation of embryonic stem cells in a mouse model of Huntington's disease

18:46h-18:54h

Anselme Perrier (Evry Cedex, France)

*Huntington's disease cell therapy in rodent:
Cell biology of human ES derived striatal graft three months after transplantation*

19:00h-19:30h

NECTAR Business Meeting

19:30h

Dinner at own expenses

8:30h-10:35h**Session III****Gene Therapy**

Chair: Deniz Kirik

8:30h-8:55h

Elisa Garcia Garcia (Madrid, Spain)*What is New? Latest Experimental Data*

8:55h-9:20h

Luciano Conti (Milan, Italy)*Novel Neural stem cell systems*

9:20h-9:45h

Jeffrey Kordower (Chicago, USA)*AAV2-Neurturin Gene Therapy for Parkinson's Disease*

9:45h-10:10h

Johan Jakobsson (Lausanne, Switzerland)*Conditional Gene Transfer to the Central Nervous System: Limitations and Possibilities*

10:10h-10:35h

Stephane Palfi (Creteil, France)*Long Term Correction of Parkinsonian Symptoms in a non Human Primate Model Using a Lentiviral-Mediated Dopamine Replacement Strategy***10:35h-11:00h**

Coffee Break

11:00h-13:00h**Session IV****Dyskinesias**

Chair: Christian Winkler (Hannover, Germany)

11:00h-11:30h

Manolo Carta (Lund, Sweden)*Dopamine release as a false neurotransmitter from striatal serotonin terminals is the prime trigger of dyskinesia in the rat 6-OHDA model*

11:30h-12:00h

Emma Lane (Cardiff, UK)*Modeling graft-induced dyskinesia in rodents*

12:00h-13:00h**Datablitz Session IV****Parkinson's Disease – Experimental Models**

Chair: Christian Winkler (Hannover, Germany)

12:00h-12:08h

Ayse Ulusoy (Lund, Sweden)*Parkin does not cause significant protection against 6-OHDA induced dopaminergic cell death*

12:08h-12:16h

Tomas Björklund (Lund, Sweden)*Optimization of in vivo levodopa production following co-administration of rAAV vectors encoding for tyrosine hydroxylase and GTP cyclohydrolase1*

12:16h-12:24h

Thomas Carlsson (Lund, Sweden)*Role of serotonin neurons in graft-induced dyskinesia in the rat model of Parkinson's disease*

12:24h-12:32h

Liselijn Wisman (Lund, Sweden)*Possible functional interaction of dopamine and acetylcholine on learning and memory at the level of the prefrontal cortex and hippocampus*

12:32h-12:40h

Claudia Ganser (Freiburg, Germany)*Neurotoxicity of 6-OHDA in vitro and neuroprotective effects of erythropoietin (EPO) and TAT-Bcl-x_L in this in vitro model of Parkinson's disease*

12:40h-12:48h

Talib Omer (Freiburg, Germany)*Investigating the potential of rat embryonic ventral mesencephalic stem cells to differentiate into dopaminergic neurons after expansion*

12:48h-12:56h

Zhigang Gong (Freiburg, Germany)*In vitro culture and induced differentiation of ventral mesencephalic precursors from rat embryos at different embryonic stages*

12:56h-13:04h

Pia Jensen (Odense, Denmark)*Expansion and dopaminergic differentiation of ventral mesencephalic precursor cells: Influence of oxygen tension***13:00h-14:00h**

Lunch Break on-site in Conference House

14:00h-16:00h**Session V****Stem Cells (1)**

Chair: Anders Björklund (Lund, Sweden)

14:00h-14:30h

Wolfgang Wurst (Munich, Germany)*Gene Regulation of the Dopamine System*

14:30h-15:00h

Ernest Arenas (Stockholm, Sweden)*Stem cell-based strategies for the treatment of Parkinson's disease*

15:00h-15:30h

Günter Höglinger (Marburg, Germany)*Adult Nigral Neurogenesis in Parkinson's Disease*

15:30h-16:00h**Datablitz Session V****Stem Cells**

Chair: Anders Björklund (Lund, Sweden)

15:30h-15:38h

Claudia Ganser (Freiburg, Germany)*In vitro differentiation of human umbilical cord blood progenitor cells*

15:38h-15:46h

Martina Maisel (Dresden, Germany)*Transcription profiling of adult and fetal human neural progenitor cells*

15:46h-15:54h

Sjef Copray (Groningen, Netherlands)*Epigenetic modification in self-renewal and differentiation of neural stem cells*

15:54h-16:02h

Maja Dieterlen (Leipzig, Germany)*Long-term in vitro transduction of human neural stem cells***16:00h-16:30h**

Coffee Break

16:30h-18:30h**Session VI****Stem Cells (2)**

Chair: Ernest Arenas (Stockholm, Sweden)

16:30h-17:00h

Philipp Koch (Bonn, Germany)*Human Embryonic Stem Cells as Donor Source for Neural Transplants*

17:00h-17:30h

Jan Pruszek (Boston, USA)*Requirements for cell sorting and cell type analysis of stem cell-derived neural cell suspensions for transplantation*

17:30h-18:00h

Ilyas Singec (La Jolla, USA)*Neural Induction of Human Embryonic Stem Cells: Overview and New Directions*

18:00h-18:30h

Uwe Himmelreich (Köln, Germany)*Stem cell tracking in in vivo animal models using Magnetic Resonance Imaging – perspectives and challenges.***20:00h****Meeting Dinner** at the "Historische Kaufhaus":

Departure at 19:45h at the Dorint Hotel reception, 10 min walk through the historical centre of Freiburg

Programme:**"Confessions"** by Pavlos Hatzopoulos

A lecture concert with the Greek pianist Pavlos Hatzopoulos are truly special musical events. Tonight he will perform and explain pieces by Bach, Beethoven, Schubert, Chopin and Moussorgsky.

Saturday

02.Dec.06

08:30h-11:00h

8:30h-10:30h

Session VII

Other Diseases

Chair: Anders Björklund (Lund, Sweden)

8:30h-9:00h

Zaal Kokaia (Lund, Sweden)

Neural Stem Cells and Stroke

9:00h-9:30h

Marco Timmer (Hannover, Germany)

Neural Progenitors as Alternative Cell Source combined with Neurotrophic Factors

9:30h-10:00h

Armin Blesch (La Jolla, USA)

Neurotrophic Factor Therapy in Alzheimer's disease

10:00h-10:30h

Martin Köllensperger (Innsbruck, Austria)

News on Multiple System Atrophy (MSA)

10:30h-11:00h

Coffee Break

11:00h-12:15h**Datablitz Session VII****Miscellaneous**

Chair: Morton Meyer (Odense, Denmark)

11:00h-11:08h

Rike Zietlow (Cardiff, UK)*Long-term expanded neurospheres fail to survive following intracerebral transplantation*

11:08-11:16h

Florian Tribl (Bochum, Germany)*The biochemistry of neuromelanin granules*

11:16-11:24h

Guilherme Lepski (Freiburg, Germany)*Neuronal differentiation of mesenchymal stem cells depends on PKA-pathway and on intracellular Ca²⁺-concentration*

11:24h-11:32h

Norbert Weidner (Regensburg, Germany)*Autologous adult neural progenitor cell transplantation represents a feasible strategy to promote structural repair in the chronically injured spinal cord*

11:32h-11:40h

Javorina Milosevic (Leipzig, Germany)*Uracil nucleotides stimulate human neural precursor cell proliferation and dopaminergic differentiation*

11:40-11:48h

Ana Sofie Correia (Lund, Sweden)*Effects of fibroblast growth factor (FGF)-20 on the differentiation of human embryonic stem cells into dopaminergic neurons*

11:48h-11:56h

Vincent Ries (Marburg, Germany)*The survival signalling kinase AKT/PKB induces trophic effects in murine models of Parkinson's disease*

11:56h-12:04h

Martin Köllensperger (Innsbruck, Austria)*Loss of dopaminergic responsivity in the double lesion SND/MSA-P rat model*

12:04h-12:12h

Grainne O`Keefe (Cambridge, UK)*A mechanism for the proliferative action of dopamine in the SVZ***12:15h-12:30h****Closing Remarks****12:30h**

Buffet lunch on-site in the Dorint Hotel

Thurs, 30.11.06

ABSTRACTS I

Pharmacotherapy in Parkinson´s Disease

P. Odin

Department of Neurology, Central Hospital, Bremerhaven, Germany

The identification of dopamine as a signal substance and the following development of L-dopa to an effective treatment for Parkinson´s disease (PD) represent one of the greatest success stories in the history of neuroscience. Also subsequently the development of new pharmacological treatment options for PD has been impressive. In spite of this many of the basic problems with the treatment remain. This concerns especially the development of motor fluctuations and dyskinesias, occurring in about 50% of the older and up to 90% of the younger patients after 5 years of treatment. It seems clear that this is an effect of pulsative dopaminergic stimulation.

Much of the recent development in the pharmacotherapy therefore now focuses on options providing a more continuous dopaminergic stimulation, CDS. In early disease stages, CDS is provided by the use of long-acting dopamine agonists, also by the use of MAO-B- and COMT- inhibitors combined with small doses of L-dopa. In late stage disease, CDS can be provided by the use of portable infusion pumps, used for subcutaneous delivery of the dopamine agonist Apomorphine or a L-dopa/Carbidopa Gel (Duodopa) in Jejunum. A further alternative is the use of high- dose dopamine agonist treatment perorally (combined with small doses of L-dopa, or no L-dopa at all). A long-term goal would be to provide CDS through normalizing the delivery of L-dopa/dopamine directly in the striatum. This would involve one or more of the surgically based techniques in focus of the present conference.

In the development of new pharmacological options the focus has been set on non-dopaminergic treatment options, but also on development of new delivery systems for the dopaminergic therapies. After the strong focus on the motor function the last decades now a new emphasis has been set on the non-motor symptomatology (NMS) of Parkinson´s disease and it has become increasingly clear that these symptoms have a high relevance for the quality of life for these patients. NMS are diverse and range from memory difficulties, low mood and depression to constipation, urinary and sexual problems and poor sleep. In the development of new treatment options, it will be crucial to closely monitor NMS and optimize the methodology with respect to this part of the symptomatology.

Deep Brain Stimulation in PD: Criteria for Patient Selection

Andreas Kupsch

The future of cell therapies in the treatment of Parkinson's disease

Roger A BARKER

*Cambridge Centre for Brain Repair & Department of Neurology, Addenbrooke's Hospital and the University of Cambridge, Cb2 2PY
Email: rab46@cam.ac.uk*

Parkinson's disease (PD) is a common neurodegenerative disorder of the CNS which is characterised by the loss of the dopaminergic neurons within the substantia nigra. This however is only part of a much greater pathological process which accounts for many of the other features of PD, including the affective, cognitive and autonomic abnormalities that are seen in significant numbers of patients. This heterogeneity of PD has become increasingly recognised, as has its basis and may in part explain some of the differing experiences with novel therapeutic approaches to treating PD such as cell transplantation.

In this respect transplantation of human fetal ventral mesencephalic tissue into the striatum of patients with PD has now been in the clinic for close to 20 years without any clear conclusions emerging on its widespread utility. Indeed some patients have experienced sustained improvement years after grafting whilst others have developed disabling graft induced dyskinesias requiring further neurosurgical intervention. In this talk I will attempt to review the reasons for the disparity in these clinical outcomes with the hope of laying out a rationale for a new cell therapy trial for PD using human fetal allografts of ventral mesencephalic tissue. This new trial will try to take into account those factors which have been identified to date as being possibly predictive of outcome including patient selection, transplant placement and preparation as well as immunosuppression. The hope is that by rigorously controlling these variables more consistent results can be obtained using this primary fetal neural transplantation approach, which will pave the way for the translation of new stem cell therapies into patients with PD.

Our own work is supported by the MRC and PDS (UK).

Co-grafting as a method for improving survival of transplanted ventral mesencephalon neurons

A.M. Szczesniak, H.A. Robertson

Brain Repair Centre, Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 1X5

Over the past 30 years, animal experimentation and open clinical trials have provided unequivocal evidence that neural transplantation is effective in Parkinson's disease (PD). However, application has been limited by the limited survival of grafted neurons, by variability in clinical outcome and by emergence of graft-induced dyskinesias. Previous studies have suggested that co-grafting supportive cells might improve graft survival. For example, co-culturing mouse ventral mesencephalon (VM) cells with mouse neural precursor cells (NPCs), but not with mouse 3T3 cells (fibroblasts) or other cell lines, was found to increase survival of dopaminergic neurons *in vitro*, an effect probably largely the result of NPC secretion of sonic hedgehog. *In vivo*, co-grafts of NPCs increase both survival and the behavioral effects of VM grafts in the 6-OHDA lesion rat model of PD (Rafuse *et al.*, 2005).

To further characterize this effect, we have investigated the effects of co-transplantation of mouse VM cells, with either mouse neural progenitor cells (NPCs) or the 3T3 fibroblast cell line, on VM graft survival in the 6-OHDA lesioned rat. Mouse 3T3 cells are a fibroblast line that was used to attempt to control for the effects of a xenograft in these immunosuppressed rats.

Rats with unilateral 6-OHDA lesions were divided into three groups and received striatal grafts, with following combinations of cell suspensions: (1) 50,000 eGFP- VM cells only, (2) 50,000 eGFP-VM + 400,000 NPCs, and (3) 400,000 eGFP-VM + 50,000 3T3. At either 2, 4 or 12-weeks post-transplantation animals were assessed behaviorally and for survival of TH neurons. To our surprise, although we had found previously that 3T3 cells had little or no effect on VM survival *in vitro* compared to the NPCs, *in vivo* the co-transplantation of VM cells with 3T3 cells significantly increases the survival of the GFP-VM grafts, as compared to VM-only or VM + NPCs groups. The number of DA neurons was higher in the eGFP-VM grafts when they were co-transplanted with either cell type, as compared to VM-only. The rotational behavioral assessment revealed that the animals that received the VM cells in combination with either NPCs or 3T3s had accelerated functional recovery.

This study reinforces the idea that co-grafting might provide a way of improving survival of dopaminergic neurons *in vivo*. It also once again raises the question of the value of *in vitro* experiments in predicting *in vivo* effects in neural transplantation.

(Supported by the Parkinson Society Canada and the Canadian Institutes of Health Research; AMS is a Fellow of the Parkinson Society Canada)

Green Fluorescent Protein (GFP) transgenic rats: A new tool for transplantation

Martin Krause, Anna Papazoglou and Guido Nikkhah

Laboratory of Molecular Neurosurgery, Dept. of Stereotactic Neurosurgery, University of Freiburg, Neurocentre, Breisacher Str. 64, Freiburg, Germany

The principal goal of neuroregenerative strategies is to restore the anatomy and the function of damaged neural circuitries. While stem cell transplantation is considered a promising therapeutic approach, knowing the fate of transplanted cells using appropriate markers is essential. GFP transgenic animals (Inoue et al, *Biochem Biophys Res Commun* 329 (2005) 288-295) express GFP ubiquitously and can be used as transplanted cell resource for monitoring donor's cellular fate during migration and differentiation phases *in vivo*.

A rat model of Parkinson's Disease (PD) is based on unilateral injections of 6-hydroxydopamine (6-OHDA) into medial forebrain bundle of the rat, resulting in a complete loss of nigral dopaminergic (DAergic) neurons and leading to a depletion of dopamine within the relevant striatum. After the lesion, primary cell suspensions rich in DAergic neurons, derived from dissociated ventral mesencephalon (VM) of E14 rat embryos, are transplanted into the lesioned striatum. Intra-striatal grafts of dopaminergic neural progenitor cells can reinnervate the striatum and restore, at least partly, lesion-induced behavioural deficits. In this study, VM cells of E14 (CRL=10-11mm) GFP Lewis rats were transplanted into the striatum of lesioned Spargue Dawley rats.

Taken in consideration the fact that the donor and the recipient belong to two different rat strains, special focus was set on the survival of the grafts, expression of GFP and how the survival correlates with the immunosuppression.

Transplanted animals were divided into two groups, one with and one without immunosuppression. Half of the animals of each group were sacrificed in two weeks after transplantation (short cell survival) and the other at four weeks (long term survival). Lesion and transplantation effects were evaluated with drug-induced rotations after 6 weeks of lesion and 2 and 4 weeks after transplantation.

This is an ongoing study and the evaluation of survival, functional and structural integration of the grafts as well as graft GFP expression analysis will be presented in the conference. However, preliminary data suggested that GFP rats might serve as an excellent tool for studying neural stem plasticity in the transplantation paradigm.

Intra-nigral grafting of foetal ventral mesencephalon from the TH-GFP mouse reveals graft-derived dopaminergic innervation of the host forebrain

L.H. Thompson, D. Kirik and A. Björklund

Wallenberg Neuroscience Center, Dept. of Experimental Medical Science, Lund, University, Sweden

Restoration of motor function in Parkinson's disease (PD) following transplantation is critically dependent on robust innervation of the host putamen by grafted dopamine neurons. At present, this requires that grafts be placed in an ectopic location directly into the putamen of the patient. Attempts to more accurately reconstruct the nigro-striatal projection system in animal models of PD, through homotopic grafting of foetal ventral mesencephalon (VM) back into the substantia nigra, have not been particularly successful in terms of restoration of motor function. Although it has been inferred that this is likely due to lack of dopaminergic innervation of the host striatum, it has been technically difficult to accurately assess the fibre outgrowth patterns of grafted dopamine neurons following intra-nigral allografting. Using foetal VM from the TH-GFP reporter mouse, we have found that dopamine neurons in intra-nigral grafts placed in adult mice or rats can indeed send long-distance projections into the host forebrain, including the most anterior aspect of the striatum. Although the degree of striatal innervation is substantially less than can be achieved through intra-striatal grafting, these results show that, in principle at least, grafted dopamine neurons can reconstruct the nigro-striatal pathway following allo-grafting into the adult brain.

In vitro differentiation of neural stem cells isolated from the adult brain

Christina Zechel ¹⁾, Christel Herold-Mende ²⁾ and Volker Tronnier ¹⁾

1) Department of Neurosurgery, Universitätsklinikum Schleswig-Holstein, University of Lübeck, Ratzeburger Allee 160, D-23538 Lübeck

2) Department of Neurosurgery, University of Heidelberg, D-69120 Heidelberg

Stem cells persist in many tissues throughout life. In the adult brain neural stem cells (NSC) are found in the subgranular zone (SGZ) of the hippocampal dentate gyrus and subventricular zone (SVZ), where they provide a lifelong source for neurons and glia. We want to find out how differentiation of NSC from the SVZ or the SGZ of the adult mammalian brain might be controlled in vitro. In particular, we like to establish strategies that would predetermine NSC for the differentiation along specific neural differentiation pathways (i.e. into dopaminergic derivatives) and thus provide neural cell derivatives that would match the requirements of specific transplantation assays (i.e. for the treatment of patients with Parkinson disease).

Since retinoic acid (RA) is a morphogen that affects development, including neurogenesis, and affects expression of the dopamine receptors D1 and D2, we studied how NSC retinoic acid would affect adult NSCs. For this purpose, we established monolayer cultures of neural progenitor cells from the dentate gyrus and the olfactory bulb of two adult patients undergoing neurosurgical operations and the SVZ and SGZ of adult rats. The cultures were characterized on the cellular and molecular level. Both, human and rat neural progenitor cells stained positive for Nestin, which is a marker for proliferating neural stem cells. Immunofluorescence analysis showed that a large amount of the NSC co-expressed Nestin and GFAP (glial fibrillary acidic protein). Both, Nestin⁺ and Nestin⁺/GFAP⁺ cells stained negative for the neuronal markers synaptophysin and MAP2 (microtubule-associated protein 2). The RA-induced changes in cell proliferation, cell morphology, marker expression and the biochemical features of NSCs will be discussed.

Thurs, 30.11.06

ABSTRACTS II

UK transplant trial of fetal striatal tissue in mild-moderate Huntington's disease – An update.

Roger A BARKER and Stephen B DUNNETT on behalf of the UK-NEST consortium

The current status of the UK transplant trial is one in which the necessary GMP facilities are awaited in order to continue transplanting the patients who have been identified for this procedure. To date five patients have received transplants bilaterally to the striatum, the first four in a staged process, the last patient had it done simultaneously. These patients have now been followed for at least three years and we shall be presenting an update on these patients but overall there have been no major improvements in any of these patients, although in some cases there is some evidence for a degree of stabilisation. In addition there are two other patients who have been transplanted within the United Kingdom at King's College Hospital in London in 1999 and 2000, and which are now followed up in Cambridge. One of these patients has not shown any significant improvement following grafting although had a complicated protracted clinical condition some 12 months after the transplant procedure. The other patient however has shown significant clinical improvement which has been sustained for 5-6 years and correlates with improved raclopride binding in the striatum on PET scanning. We shall present an update on these patients which are now followed up in Cambridge with the other grafted patients, as well as briefly discussing the current legislative factors limiting the progress of this trial at the present time.

This work was supported in part by the MRC.

Effect of fetal neural transplants in patients with Huntington's disease 6 years after surgery: a long-term follow-up study.

A. Bachoud-Lévi, V. Gaura, P. Brugières, J. Lefaucheur, M. Boissé, P. Maison, S. Baudic, M. Ribeiro, C. Bourdet, P. Remy

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BACKGROUND: Although we have shown in three out of five patients with Huntington's disease that motor and cognitive improvements 2 years after intracerebral fetal neural grafts are correlated with recovery of brain metabolic activity in grafted striatal areas and connected regions of the cerebral cortex, neural grafts are not known to have protective effects on the host brain per se. We undertook long-term follow-up of previously reported patients with the disease to ascertain the nature and extent of any secondary decline after grafting. **METHODS:** Five patients with Huntington's disease from our pilot study were assessed annually with the unified Huntington's disease rating scale, neuropsychological tests, and MRI, for up to 6 years after neural grafting. Resting cerebral activity was recorded at 2 and 6 years. **FINDINGS:** Clinical improvement plateaued after 2 years and then faded off variably 4-6 years after surgery. Dystonia deteriorated consistently, whereas chorea did not. Cognitive performance remained stable on non-timed tests, whereas progression of motor disability was shown by deterioration on timed tests. Hypometabolism also affected the brain heterogeneously, sparing the benefits in the frontal cortex and at the precise location of the grafts, but showing a progressive deterioration in other areas. Two patients who had no benefit from grafting at 2 years continued to decline in the same way as non-grafted patients. **INTERPRETATION:** Neuronal transplantation in Huntington's disease provides a period of several years of improvement and stability, but not a permanent cure for the disease. Improvement of the surgical procedure and in patient selection could improve the therapeutic value, but neuroprotective treatment seems to be unavoidable in the disease.

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Transplantation of fetal striatal cells in patients with Huntington's disease (HD): the German experience within a European-based multi-center study

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The implantation of neural progenitor cells for the treatment of neurodegenerative disorders like HD or PD is a powerful neurobiological tool, as shown experimentally over the last two decades. However, its clinical potential, efficacy and safety for neurodegenerative diseases like HD have still to be fully elucidated. In this study the centers in Freiburg and Ulm are part of a multi-center, randomised and controlled Phase II transplantation study in HD. Seven European centres are enrolled with Freiburg being the transplant centre in Germany. 80 HD patients in total will be transplanted with fetal striatal cells of which 20 patients will be transplanted in Germany. The randomisation procedure built into the study protocol allow for both a longitudinal and horizontal comparison of neural graft effects.

Inclusion criteria are clinically symptomatic and genetically confirmed Huntington Disease (number of CAG repeats ≥ 36), between 25 and 65 years of age, early to moderate stage of the disease (UHDRS motor ≥ 5), preserved autonomy, TFC (Total Functional Capacity) > 9 . Exclusion criteria are advanced disease, precluding the ability to give informed consent, very early stage of disease causing minor disability, severe comorbidity that could compromise the life prognostic or preclude general anaesthesia or immunosuppression, Mattis Dementia Rating Scale < 120 , psychiatric or personality disturbances that might compromise the follow-up, participation at another trial, severe cortical atrophy seen on CT and MRI, lack of HD specific changes in T2 sequence of MRI, positive HIV serology (HIV1, HIV2, AgP24), active hepatitis (B and C), HTLV 1 and 2.

All patients (group 1 after 6 months and group 2 after 26 months) receive a stereotactic placement of human fetal striatal cells into the head of the caudate nucleus (2 trajectories) and into the putamen (3-4 trajectories) into both hemispheres. Postoperatively, patients are treated with a triple immunosuppressive therapy (cyclosporine, azathioprine and prednisolone) which is discontinued 6 – 12 months after the second grafts and monitored by routine blood test. The patients are monitored six months preoperatively and at least 36 months postoperatively by a battery of clinical, neuropsychological, psychiatric, and neurophysiological and neuroimaging (MRI and PET) tests. Primary endpoints are progression on the motor UHDRS scale and 11C-Raclopride-PET and 18F-FDG-PET pre-op and 18 months post transplantation.

Until now we have transplanted five patients in group 1 and followed five patients in group 2 and in the latter group (after 2 years further follow up) the first patient received his first transplant recently. There were no perioperative complications and one patient committed suicide 6 months post transplantation (group 1) and his brain was carefully evaluated by immunohistochemical analysis. Further clinical and neuroradiological data will be provided to discuss the potentials and limitations of the current transplantation approach for HD.

Human foetal striatal transplantation in Huntington's disease. First Italian clinical trial at the University of Florence: Preliminary report

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In February 2006, after approval of the National Ethics Committee, the first Italian clinical program of neurotransplantation was started at the Florence University. Four clinically and genetically defined Huntington's disease (HD) patients underwent human fetal striatal transplantation (HFST). Patient 1 and 2 received bilateral graft in two sessions 1 month apart, while patient 3 and 4 have not received the contralateral graft yet. Small blocks of tissue, obtained from the whole ganglionic eminence of 9–12 weeks post-conception fetus, were processed to obtain cell suspension and then implanted in the head of the caudate nucleus, in the pre and possibly in the post-commissural putamen by means of robotic-assisted-stereotactic procedure. We used only one fetus donor for each procedure. Immunosuppression was started one day before the first graft with cyclosporin A, prednisolone and azathioprine. The procedure resulted feasible and well tolerated in all cases. Follow-up ranged from 2 weeks to 8 months. Patient 2 experienced, one month after each graft, the occurrence of subdural haematoma necessitating surgical evacuation. No serious adverse events were observed in the remaining patients. Neurologically, patient 1 remained stable and patient 2 showed a slight improvement of chorea and of the daily activity performances. Patients 3 and 4 do not have a sufficient follow-up. In all patients magnetic resonance (MR) imaging did not detect any complication and showed the needle tracks within the appropriate anatomical targets. On patient 1 MR examination, a signal conspicuously growing over time in the region of grafts appeared. At the 6 months imaging control of the same patient, two solid not enhancing nodular areas with almost the same signal of the grey matter, grossly respecting the morphology of basal ganglia, were detected in the right striatum. Longer follow-up and larger number of patients are needed to assess efficacy of HFST in HD.

Histological evaluation of six months post implantationem striatal fetal stem-cell grafts in a Huntington's disease patient: Signs of ongoing mitosis, neurogenesis, beginning maturation and integration

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Foetal stem-cell (FSC) transplantation is one promising contemporary therapeutic approach that could halt or probably reverse the fatal course of Huntington's disease.

Despite manifold positive observations in animal models, histological data of the engrafting procedure in human brain remains sparse, though especially knowledge about the time course and extent of maturation, as well as the amount of graft rejection could affect therapeutic and diagnostic considerations.

Here we report about the immunohistological evaluation of human striatum six months after stereotactic implantation of human embryo derived tissue from the ganglionic eminence, concerning maturation and immunological host-response.

The grafts are still showing strong mitotic activity, immature phenotype, heavy migration into host tissue and beginning maturation and integration of graft derived neurons, as shown by immunofluorescence and combinations of immunohistochemistry and fluorescence in-situ hybridization, though certain subtypes of striatal neurons are missing and complete adult phenotype is not reached at all.

Immunologic host response, despite administered immuno-suppressants, reaches locally, especially around vessels, considerable levels, yet viability of engrafted cells in these areas remains good.

In conclusion, our morphological evaluation of a transplanted Huntington's patient brain confirms good graft survival and ongoing, albeit yet not terminal, differentiation six months after transplantation.

The extracellular matrix molecule tenascin-R enhances neuronal differentiation of embryonic stem cells in a mouse model of Huntington's disease.

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Huntington's disease is a neurodegenerative movement disorder of the central nervous system that is associated with a degeneration of striatal GABAergic interneurons. Since current treatments can be regarded as merely symptomatic, research on alternative therapeutic approaches needs to be pursued. Transplantation of genetically modified stem cells into lesioned brain areas of patients is a possible alternative. In our study we have used green fluorescent protein-labeled murine embryonic stem cells stably transfected to overexpress the extracellular matrix molecule tenascin-R under the control of the universal PGK-promotor and transplanted these cells after pre-differentiation *in vitro* into the striatum of quinolinic acid-treated mice, a model for Huntington's disease. Tenascin-R is expressed by myelinating oligodendrocytes and remains expressed at nodes of Ranvier in the adult. It is also expressed in perineuronal nets surrounding inhibitory interneurons in many brain regions including the striatum. When ectopically expressed by fibroblasts grafted into the striatum of adult mice it attracts stem cells from the rostral migratory stream. We found that in comparison to sham-transfected control cells, tenascin-R overexpressing embryonic stem cells showed enhanced differentiation into neurons and reduced differentiation into glial cells *in vitro* and generated more neurons and fewer astrocytes one month and two months after transplantation. Differentiation into inhibitory versus excitatory neurons was not affected by tenascin-R overexpression. Furthermore, tenascin-R overexpressing stem cells showed reduced migration in a scratch assay *in vitro* and reduced migration in the host striatum *in vivo*. These findings indicate that tenascin-R overexpressing and pre-differentiated embryonic stem cells exert both beneficial effects towards neuronal differentiation, but unfavourable effects with regard to migration *in vitro* and *in vivo*. However, neither tenascin-R overexpressing nor non-overexpressing stem cells exerted a positive influence on locomotor recovery when compared to vehicle solution treated control animals.

Huntington's disease cell therapy in rodent: Cell biology of human ES derived striatal graft three months after transplantation.

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Huntington's disease (HD) is a neurodegenerative monogenic disease affecting 1/10000 adults in Europe. The symptoms are associated with preferential degeneration of the medium size spiny GABA neurons (MSN) of the striatum, MSN are DARPP-32+ and constitute over 90% of the entire neuronal population of the striatum.

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. Recent advances with neural differentiation protocols of human embryonic stem cells (hESC) have shown that hESC lines are prime candidates to provide an unlimited source of striatal progenitors suitable to replace efficiently fetal graft for HD therapy.

We adapted a stromal feeder based differentiation protocol of hESC to generate neural progenitors and neurons enriched in striatal cells. Both real time PCR analyses and immuno-fluorescence staining of key markers of neural, neuronal and striatal cells showed the selective enrichment of our culture in striatal-committed cells. Following this protocol, neural culture were produce at different stage of differentiation in order to determine their therapeutic potential *in vivo*.

A first series of transplantations were aimed at the short term assessment (4-6 weeks) of the proliferation and neural commitment properties of these grafts. A battery of histological tests was performed to characterize the graft. Special emphasis was placed, in the demonstration of the full commitment of transplanted cells to the neural lineage, as opposed to the formation of teratoma. A second series of transplantations were aimed at the long term assessment (3+ months) of the cellular biology of selected grafts. In particular, we examined the proliferation properties of these grafts as well as their striatal maturation and anatomical integration inside a striatum experimentally depleted in GABA neurons.

Fri, 01.12.06

ABSTRACTS III

What is new? Latest experimental data

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Little progress has been made in the context of developing therapies for human Central Nervous System diseases since the use of fresh foetal tissue grafting four decades ago until the latest human ES research, passing through the use of all source of progenitor/precursor/stem neural cells.

Nowadays, the main road-blocks in cell therapy research are a) how to limit the proliferation of embryonic stem cells; b) how to extend the proliferative capacity of foetal and adult neural stem cells; c) how to instruct embryonic stem cells to differentiate into the desired neural lineage and not in other cell types; d) how to get stem cell derivatives to reach the desired mature functional phenotype once they are grafted.

In recent years, investigating how to generate functional human dopaminergic neurons for cell replacement in Parkinson's disease (PD) models, we found that the Bcl-X_L protein was able to enhance the capacity of forebrain and midbrain human neural stem cells to generate human dopaminergic neurons. We then investigated the actions of Bcl-X_L in these hNSCs preparations, finding that Bcl-X_L increases the overall neuron production, however the proportion of dopaminergic neurons was enriched comparing the rest of phenotypes. We also find that the increase in neurons is due to a higher proliferation of neuronal progenitor. Moreover, we observed a profound decrease in glia production. This higher production of neurons and glia reduction was also observed in transplants of Bcl-X_L overexpressing human NSCs in aged rats.

In another context, research in our group in recent years has aimed to the development of homologous recombination procedures to genetically modify human NSCs. Homologous recombination not only offers a precise, site-specific way for genetic modification, but also let us choose between house-keeping (stable/continuous) or regulated expression of the transgene. New data from our laboratory get us closer to "the desired recombinant".

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Novel neural stem cells systems

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Classically, neural stem and progenitor cells are derived from neural tissue and cultured in aggregates known as neurospheres. However, the proportion of 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, an homogeneous source of neural stem cells is represented by pluripotent mouse embryonic stem (ES) cells that expand by symmetrical divisions in adherent monoculture. Differently from ES cells, homogenous expansion of somatic tissue-restricted stem cells has proven elusive. Here we describe derivation and homogenous propagation of adherent neural stem (NS) cells both from ES cells via neural lineage commitment of ES cells followed by growth factor addition in basal culture conditions. NS cells proliferate continuously in the presence of growth factors, are diploid, and clonogenic. After prolonged expansion, they remain able to differentiate efficiently into neurons and astrocytes in vitro and upon transplantation into the adult brain. NS cells uniformly express morphological and molecular features of radial glia, developmental precursors of neurons and astrocytes. Consistent with this profile, equivalent adherent NS cell lines can readily be established from fetal mouse brain. 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.

AAV2-Neurturin Gene Therapy for Parkinson's Disease

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Gene delivery of trophic factors such as neurturin hold promise in providing a disease modifying therapy, in addition to symptomatic therapy for patients with Parkinson's disease. This presentation will focus on the following findings:

In intact monkeys, AAV-NTN (CERE-120) increases striatal dopamine and nigral TH in a dose dependent manner.

In intact monkeys AAV-NTN is retrogradely transported and activates phospho-ERK in a dose dependent manner

In aged monkeys, AAV-NTN (CERE-120) reverses age-related declines in dopamine as measured by fluorodopa-PET and post-mortem TH.

In aged monkeys, intrastriatal AAV-NTN (CERE-120) activates phospho-erk in the nigral perikarya.

In MPTP treated monkeys, AAV-NTN (CERE-120) prevents the emergence of parkinsonian signs for up to 10 months.

In MPTP treated monkeys, AAV-NTN (CERE-120) attenuates the loss of striatal dopamine.

In MPTP treated monkeys, AAV-NTN (CERE-120) prevents the loss of nigral neurons.

Based upon these and other data, Ceregene inc. has initiated a Phase 1 clinical trial testing the safety of AAV delivery of CERE-120 in patients with Parkinson's disease and the design and results from this trial will be briefly discussed.

Conditional gene transfer to the central nervous system: Limitations and possibilities

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The ability to control and regulate the level of transgene expression following gene delivery may turn out to be crucial for several gene therapy strategies. For this purpose a number of transgenic "switches" have been developed. Examples of such switches are the tetracycline and rapamycin inducible systems as well as various strategies utilizing the Cre recombinase. In recent years these systems have been greatly improved and now allow highly efficient drug-controlled gene expression. This talk will provide an overview of these inducible systems for conditional gene transfer and review their potential usefulness in the central nervous system. Recent data of our own experience with the tetracycline system will also be presented. Finally, the possibility to transfer these technologies to the clinical situation will be discussed.

Long Term Correction of Parkinsonian Symptoms in a non Human Primate Model using a Lentiviral-Mediated Dopamine Replacement Strategy

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Fri, 01.12.06

ABSTRACTS IV

Dopamine release as a false neurotransmitter from striatal serotonin terminals is the prime trigger of dyskinesia in the rat 6-OHDA model

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The serotonin neurons are known to be able to convert exogenous L-DOPA to DA, and store and release DA in an activity-dependent manner (Ng et al., 1970; Ng et al., 1971; Arai et al., 1994; Tanaka et al., 1999). L-DOPA-derived DA acting as a "false transmitter" in serotonergic neurons may be particularly important in advanced stages of the disease when a major part of the nigrostriatal DA system has degenerated and the remaining DA neurons are in a compromised functional state (Miller and Abercrombie, 1999; Tanaka et al., 1999; Kannari et al., 2001). However, the efficacy of L-DOPA medication is gradually lost over time in Parkinson's disease, and abnormal involuntary movements, dyskinesias, gradually emerge as a severe side-effect in response to previously beneficial doses of the drug.

In this study we provide evidence that dyskinesias induced by chronic L-DOPA treatment in rats with 6-hydroxydopamine induced lesions of the nigrostriatal dopamine pathway is critically dependent on the integrity and function of the serotonin neuron system. Removal of the serotonin afferents, or dampening of serotonin neuron activity by 5-HT_{1A} and 5-HT_{1B} agonists, resulted in a virtually complete suppression of the L-DOPA-induced dyskinesias, suggesting that dysregulated dopamine release from serotonin terminals is the prime trigger of dyskinesia in the rat Parkinson model. In animals with complete dopamine lesions the spared serotonin innervation was unable to sustain the therapeutic effect of L-DOPA at the cylinder test, suggesting that dopamine released as a "false transmitter" from serotonin terminals is detrimental rather than beneficial. Conversely, in our experimental conditions, the therapeutic effect of L-DOPA appears dependent on the presence of some spared striatal dopaminergic terminals; indeed, partial lesioned rats significantly improved their performance in the cylinder test after L-DOPA treatment, even when the serotonergic system was removed or pharmacologically suppressed.

References

- Arai R, Karasawa N, Geffard M, Nagatsu T, Nagatsu I (1994) Immunohistochemical evidence that central serotonin neurons produce dopamine from exogenous L-DOPA in the rat, with reference to the involvement of aromatic L-amino acid decarboxylase. *Brain Res* 667:295-299.
- Kannari K, Yamato H, Shen H, Tomiyama M, Suda T, Matsunaga M (2001) Activation of 5-HT(1A) but not 5-HT(1B) receptors attenuates an increase in extracellular dopamine derived from exogenously administered L-DOPA in the striatum with nigrostriatal denervation. *J Neurochem* 76:1346-1353.
- Miller DW, Abercrombie ED (1999) Role of high-affinity dopamine uptake and impulse activity in the appearance of extracellular dopamine in striatum after administration of exogenous L-DOPA: studies in intact and 6-hydroxydopamine-treated rats. *J Neurochem* 72:1516-1522.
- Ng KY, Colburn RW, Kopin IJ (1971) Effects of L-dopa on efflux of cerebral monoamines from synaptosomes. *Nature* 230:331-332.
- Ng KY, Chase TN, Colburn RW, Kopin IJ (1970) L-Dopa-induced release of cerebral monoamines. *Science* 170:76-77.
- Tanaka H, Kannari K, Maeda T, Tomiyama M, Suda T, Matsunaga M (1999) Role of serotonergic neurons in L-DOPA-derived extracellular dopamine in the striatum of 6-OHDA-lesioned rats. *Neuroreport* 10:631-634.

Modelling graft-induced dyskinesia in rodents

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Clinical trials have demonstrated the potential of transplantation of embryonic ventral mesencephalon as a therapeutic approach for Parkinson's disease. However, progress has been hampered by the appearance of dyskinesia, both 'on' and 'off' concurrent L-dopa treatment, in the post-surgery interval in some patients. In order to circumvent this side effect in future cell-based therapies, the impact of different variables of transplantation on the presentation of dyskinesia are being examined. A viable animal model of L-dopa induced dyskinesia (LID) has been developed in the 6-OHDA lesioned rat, and two pharmacological approaches are now being used to examine post-transplantation dyskinesia. First, detailed determination of the effects of cell implantation on the classic LIDs demonstrates that they are in general reduced by the presence of a graft but that more hyperkinetic and choreic aspects of the movements may be exacerbated^{1,2}. Secondly, amphetamine administration triggers dyskinetic behaviours in 6-OHDA lesioned rats post-transplantation, in addition to the well known rotational response. Both the size and location of the graft has been shown to influence the severity of amphetamine-induced dyskinesia^{3,4}. We have also examined the role of host and graft-derived 5-HT and the influence of neuro-inflammation. By contrast with the success achieved in modelling LIDs and the effects of grafts on the drug-induced syndrome, it has proved more difficult to re-create the spontaneous 'off' medication dyskinesia experienced by patients.

Significant progress is being made in understanding why some transplants patients develop dyskinesias. However, there remain a number of issues highlighted by retrospective analysis of transplanted patients that remain unresolved, including the role of prior exposure to L-dopa and ongoing degeneration in non-grafted areas. The animal model of LIDS are making a significant contribution to understanding the underlying biological mechanisms, necessary to optimise not just the efficacy of cell transplantation for Parkinson's disease, but also the elimination of adverse effects.

¹Steece-Coller *et al.*, Embryonic mesencephalic grafts increase Levodopa-induced forelimb hyperkinesias in parkinsonian rats *Mov.Disord.* 18:1442-54; 2003

²Maries *et al.*, Focal not widespread grafts induce novel dyskinetic behavior in parkinsonian rats. *Neurobiol. Dis.* 21:165-80; 2005

³Carlsson *et al.*, Graft placement and uneven pattern of reinnervation in the striatum is important for the development of graft-induced dyskinesia. *Neurobiol. Dis.* 21:657-68; 2006

⁴Lane *et al.*, The impact of graft size on the development of dyskinesia following intrastriatal grafting of embryonic dopamine neurons in the rat. *Neurobiol Dis.* 22:335-45; 2006

Parkin does not cause significant protection against 6-OHDA induced dopaminergic cell death

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Mutations in several genes have been linked to familial forms of Parkinson's disease (fPD). The most common form of fPD is caused by mutations in the parkin gene, which is an ubiquitin proteasome E3 ligase enzyme. As the mutations result in an autosomal recessive juvenile form of PD, it is believed that the main pathological event is a loss of parkin function in the cells. Thus, it is conceivable that increased parkin function may provide a novel neuroprotective strategy for treatment of PD patients. It is thus important to examine if parkin overexpression can alleviate toxicity mediated by various insults in animal models. In this study our aim was to assess the neuroprotective effect of wild-type or non-cleavable (NC) parkin (constitutively active) against 6-OHDA induced dopaminergic cell death. Additional groups of animals receiving a disease causing mutant form of parkin (mut-parkin) or the GFP marker gene were used as controls. rAAV2 vectors expressing these genes were injected in the right substantia nigra. Three weeks later, half of the animals in each group received 3 injections of 7 μ g 6-OHDA in the right striatum. On week 6, cylinder and amphetamine induced rotation tests were performed. All parkin groups had a trend of higher cylinder score than the control group, but this effect was not statistically significant. Lesioned animals also failed to show significantly different amphetamine induced rotations. Animals were perfused on week 7 and brains were stained for TH, GFP and parkin. Quantification of surviving TH positive cells in the SN indicated that there was overall no statistically significant protection of DA neurons against 6-OHDA toxicity in lesioned animals.

Optimization of in vivo levodopa production following co-administration of rAAV vectors encoding for tyrosine hydroxylase and GTP cyclohydrolase 1

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Parkinson's disease (PD) is characterized by progressive loss of dopaminergic neurons within the substantia nigra. Oral administration of L-DOPA remains the gold-standard treatment for PD. However, as the disease progresses, L-DOPA induces debilitating side effects such as wearing-off, motor fluctuations, and dyskinesias. It has been hypothesized that L-DOPA-related complications may, at least in part, be due to the intermittent, pulsatile supply of L-DOPA provided by peripheral administration. Thus, novel gene therapy strategies to provide long-term continuous DOPA administration, are currently being developed. This approach is based on the co-expression of tyrosine hydroxylase (TH), the rate-limiting enzyme in the dopamine production, and GTP-cyclohydrolase (GCH1), the primary synthetic enzyme for tetrahydrobiopterin (BH4) which is an essential co-factor for TH activity. So far, all studies have used an equal mixture of two vectors, each coding for one of the two enzymes. However, as GCH1 is a stable enzyme with more favorable kinetics than TH, it is likely that a different vector ratio would result in a more efficient DOPA production. Therefore, with vectors encoding for TH or GCH1 proteins, ratios between 1:1 to 1:0.03 were investigated to determine the DOPA production rate in vivo. We found that ratios of at least 1:0.25 between TH and GCH1 vectors provide high DOPA levels and that even thirtytwo-fold dilution of the GCH1 vector result in only a 50% reduction in DOPA levels suggesting that the biopterin levels are saturated. Secondly, the data obtained from this experiment will help us to define exactly how much suppression of GCH1 will be necessary to turn off striatal DOPA production when regulated vectors are used.

Role of serotonin neurons in graft-induced dyskinesia in the rat model of Parkinson's disease

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Transplantation of fetal ventral mesencephalic (VM) neurons into the striatum of patients with advanced Parkinson's disease (PD) has been used as a restorative therapy. However, the results have been highly variable. Some patients responded well with significant improvement in motor functions, while others have not improved or even worsened in their response to L-DOPA. The variation between different trials and patients has been proposed to be, at least partly, due to the preparation of the cells, where tissue clumps, tissue stripes or single cell suspensions were used. In addition, the transplanted tissue is known to contain other cell types than dopamine (DA), such as serotonin neurons. In fact, serotonin cells can act as a potent decarboxylation and storage site for DA after L-DOPA administration. The present experiment was designed to evaluate the effect of serotonin transplants in a model of PD, where rats were rendered dyskinetic by daily chronic L-DOPA injections, resembling peak-dose dyskinesias seen in PD patients. The animals were then balanced in groups and transplanted with 130.000 cells derived from the VM. The borders for the dissection of the tissue piece was adjusted to provide cell suspensions containing serotonin or DA neurons, or in a third group a mixture of the two (standard VM grafts). Sham-operated animals served as dyskinetic or L-DOPA-naive lesion control groups. Our data show that serotonin transplants significantly increase the L-DOPA-induced dyskinesias (175% of pre-transplantation value), where the axial, dystonic, component, in particular, increased by 220% at 16 weeks after transplantation. These data suggest that grafts that contain large number of serotonin neurons (and only few surviving DA neurons), may have a detrimental effect in the induction of L-DOPA-induced dyskinesia, and contribute to the results seen in some clinical trials.

Possible functional interaction of dopamine and acetylcholine on learning and memory at the level of the prefrontal cortex and hippocampus

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Parkinson's disease (PD) is characterized by a loss of dopamine (DA) producing neurons. The main clinical symptoms are motor deficits. However, in some PD patients a cognitive decline can occur. It is not clear whether this is due to a loss of DA, primarily in non-striatal forebrain areas, or can be explained by a co-existing dysfunction of the cholinergic system, or an interaction of both. In this study we have investigated if performance in learning and memory tasks require a convergence of DA and acetylcholine at the hippocampal or frontal cortical levels. For this purpose, we lesioned the cholinergic neurons with 192 IgG-saporin in these structure and the DA neurons in the ventral tegmental area (VTA) with 6-OHDA. The cholinergic neurons of the nucleus basalis magnocellularis (NBM) project mainly to the prefrontal cortex, while the neurons of the septum project mainly to the hippocampus. Dopaminergic neurons in the VTA have projections to both structures. Five weeks after surgery, the animals were assessed for deficits in the Morris water maze test. A significant increase in latency to find the platform was found in animals with a VTA lesion but not in the NBM or septum lesioned animals. All the double lesion groups had the same increase in latency as the VTA lesioned animals. Our findings suggest that the DA neurons in the VTA have an important function in learning and memory. However our data does not point to any clear indication whether an interaction between DA and acetylcholine neurons exists.

Neurotoxicity of 6-hydroxydopamine (6-OHDA) in vitro and neuroprotective effects of erythropoietin (EPO) and Tat-Bcl-x_L in this in vitro model of Parkinson's disease

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6-OHDA is a neurotoxin commonly used to cause lesions of the dopaminergic pathways and thus represents an experimental model for Parkinson's disease both *in vivo* as well as *in vitro*. In this study 6-OHDA incubation on rat E14 (CRL=10-11 mm) ventral mesencephalon (VM) cell cultures was analysed under different culture conditions and used as an *in vitro* model for assessment of potential neuroprotective effects of EPO and Tat-Bcl-x_L.

VM cultures were incubated with 10, 15, 30 and 60 µM 6-OHDA for 1, 3, 6, 12, and 24 hours, or were pre-incubated for 4 days with EPO or 4 hours with Tat-Bcl-x_L before adding 30µM 6-OHDA for 3 and 6 hours. Afterwards cells were cultured for 2 more days before being analysed by immunohistochemistry.

Neurotoxic effects of 6-OHDA depended on time and concentration. Long term incubation resulted in a strong unspecific effect and a decrease of the total cell number, but also a clear loss of the neuronal population including the dopaminergic cells. Short incubation times combined with low concentrations did not induce a significant cell loss. Neuroprotection experiments with EPO showed a significant survival for all neuronal cells resulting in higher βTubulinIII⁺ cell percentages compared to cultures without EPO, and a significantly protective effect on dopaminergic cells when incubated with 30 µM 6-OHDA for 3 hours. Experiments with Tat-Bcl-x_L showed some significant protection if cells were incubated with 30 µM 6-OHDA for 3 hours.

These results show that longer incubation times with 6-OHDA yield in an unspecific toxic effect that cannot be prevented by the application of EPO or Tat-Bcl-x_L. The neuroprotective effects of these substances could be demonstrated when the "lower" 6-OHDA toxicity is affecting predominantly the apoptotic pathway. This provides some interesting and novel insights into the mechanisms governing 6-OHDA-induced neurotoxicity and potential neuroprotective pathways.

Investigating the potential of rat embryonic ventral mesencephalic stem cells to differentiate into dopaminergic neurons after expansion

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Neural precursor cells from the embryonic ventral mesencephalon (VM) tend to lose their developmental potential to differentiate into dopaminergic (DAergic) tyrosine hydroxylase (TH) positive neurons in *vitro* after expansion.

The aim of the study was to evaluate in *vitro* parameters and techniques to promote DA survival, differentiation and, at a later stage, functional integration.

VMs of E12 (CRL=6-7mm) and E14 (CRL=10-11mm) Spargue Dawley rat embryos were removed and dissociated. Cells were plated at a concentration of 25,000 cells/cm² in 48-well precoated with 0.01% poly-L-ornithine (PLO) plates and expanded as a monolayer or as neurospheres in untreated cell culture flasks in medium containing FGF2, EGF and heparin. The monolayer cultures were expanded for one week and differentiated for one more week in medium containing FCS and ascorbic acid. Neurospheres expanded cells were passaged once per week mechanically or enzymatically for three weeks. Every week passaged cells were plated on PLO coated plates and either immediately or differentiated for one week. Immunofluorescence staining for TH and β -Tubulin III were used to analyze and characterize the developmental stage of the cells as well as their neuronal differentiation. A detailed analysis of the proliferation and of the differentiation profiles of rat embryonic E12 and E14 cells will be presented at the NECTAR meeting demonstrating that in *vitro* expansion protocols indeed exert a significant influence on DA survival and differentiation.

In vitro culture and induced differentiation of ventral mesencephalic precursors from rat embryos at different embryonic stages

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Different ventral mesencephalic precursors from rat embryos were cultured *in vitro*, in order to determine the optimal candidates for subsequent large-scale cell transplantation as a treatment of Parkinson's disease. These precursors were collected from rat embryos at different embryonic stages which were strictly defined by the crown-rump lengths (CRLs) ranging from 3-4mm, 5-6mm, 7-8mm, 9-10mm to 11-12mm. After these precursors were treated with bFGF for 7 days in the proliferation stage, and differentiated for the next 7 days in the special differentiation medium containing L-ascorbic acid-2-phosphate sesquimagnesium salt (AA-2P), the yields of dopaminergic neurons (DNs) were detected and compared by means of immunofluorescence staining. The total number of cells in the 3-4mm culture, 5-6mm culture, 7-8mm culture, 9-10mm culture and 11-12 mm culture increased about 76.39, 59.67, 51.85, 14.35 and 10.31 times, respectively. The ratio of DNs versus the total cell population in 5-6mm culture, 3-4mm culture and 7-8mm culture decreased sequentially and significantly, but all of them were significantly higher than those found in the 9-10mm and 11-12mm embryo cultures. The percentage of DNs was significantly higher in the 5-6mm culture, compared to the 3-4mm and 7-8mm cultures, while the latter two were also significantly higher than those in the 9-10mm and 11-12mm embryo cultures. Therefore, the yield of ventral mesencephalic dopaminergic precursors is greatest from rat embryos with a CRL of 5-6mm, and these findings may promote the current scientific attempts to develop progenitor cell-based neural transplantation approaches.

Expansion and dopaminergic differentiation of ventral mesencephalic precursor cells: Influence of oxygen tension

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Methods for expansion of dopaminergic precursor cells are of interest in the search for transplantable neurons for cell therapy in Parkinson's disease. Here we investigated the influence of FGF2 and FGF8 on expansion of such precursor cells cultured at high (20%) and low (3%) oxygen tension.

Tissue from embryonic day 12 rat ventral mesencephalon was mechanically dissociated and cultured for 4 days in serum-free medium in the presence of FGF2 or FGF8 (both 20 ng/ml) or without mitogens (control). After mitogen withdrawal and addition of serum, cells were differentiated for 6 days.

Following expansion at high oxygen, significantly more cells incorporated BrdU in cultures exposed to FGF2 (51 fold; $p < 0.001$) and FGF8 (13 fold; $p < 0.05$) than in control cultures. After differentiation at the same oxygen tension, HPLC analysis revealed significantly more dopamine in the medium of FGF2-expanded (4.1 ± 0.4 pmol/ml; $p < 0.001$) and FGF8-expanded cultures (2.6 ± 0.1 pmol/ml, $p < 0.05$) than in controls (1.1 ± 0.3 pmol/ml). Correspondingly, numbers of tyrosine hydroxylase-immunoreactive (TH-ir) cells had increased significantly in FGF2-expanded and FGF8-expanded cultures. Expansion at low oxygen tension significantly increased the BrdU incorporation in both FGF2 (1.7 fold; $p < 0.01$) and FGF8 (3.0 fold; $p < 0.001$) cultures, but only FGF2-expanded cultures contained significantly more TH-ir neurons (2.2 fold; $p < 0.001$) following differentiation at low as compared to high oxygen tension. Cultures both expanded and differentiated at low oxygen tension contained more TH-ir neurons than cultures only expanded or differentiated at low oxygen. Interestingly, the low oxygen tension appeared to inhibit TH enzyme activity as reduced levels of dopamine/TH-ir cell were detected in the culture medium. However, this effect could be reversed following two days at high oxygen tension.

We conclude that the procedures employed in this study constitute an efficient method for expansion of dopaminergic precursors.

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ABSTRACTS V

Gene regulation in the dopamine system

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Midbrain dopaminergic (mDA) neurons play a pivotal role in the control and modulation of different brain functions, and their degeneration or dysfunction leads to severe neurological and psychiatric disorders, among them Parkinson's Disease (PD). Therapeutic strategies for PD include the regeneration of the degenerating mDA neurons. Therefore, understanding the normal development of this cell population is of high clinical interest.

Despite the importance of mDA neurons, the molecular mechanisms controlling their generation *in vivo* are still poorly understood. During embryogenesis, mDA neurons are specified in the ventral midbrain rostral to the mid/hindbrain boundary (MHB). We have shown previously that the position of the MHB determines the location and size of the mDA cell population *in vivo* (Brodski et al., 2003). Fgf8, a factor secreted at the MHB, and Shh, secreted from the ventral midline of the neural tube, are necessary and sufficient for ectopic induction of mDA neurons in rat embryo explant cultures. The secreted glycoprotein Wnt1 is expressed in close vicinity to developing mDA neurons in the ventral midbrain of the mouse embryo. Using different gain-of-function and loss-of-function mouse mutants, we have shown that Wnt1 controls a genetic network including *Otx2* and *Nkx2-2* leading to the establishment of the mDA progenitor domain during early neural development (Prakash et al., 2006). In addition, Wnt1 is required for the terminal differentiation of mDA neurons at later stages (Prakash et al., 2006). Wnt5a is another member of the Wnt family expressed in the ventral midbrain in an overlapping domain with the developing mDA neurons. Wnt5a was shown to promote the differentiation of mDA neurons *in vitro*. Inactivation of *Wnt5a* in mice, however, does not result in a loss of the mDA cell population but rather in an increase of the total number of mDA neurons in the ventral midbrain. In addition, two homeodomain transcription factors are required for the generation of mDA neurons: Lmx1a and Msx1 (Andersson et al., 2006). Both factors are expressed in the ventral midbrain and appear to be downstream of the Shh- and Wnt-signalling pathways in mDA neuron generation. Thus, an intricate genetic network is active during the specification of the mDA neuronal phenotype in the ventral midbrain.

References:

Andersson, E., Tryggvason, U., Deng, Q., Friling, S., Alekseenko, Z., Robert, B., Perlmann, T. and Ericson, J. (2006). Identification of Intrinsic Determinants of Midbrain Dopamine Neurons. *Cell* 124, 393-405.

Brodski, C., Weisenhorn, D. M., Signore, M., Sillaber, I., Oesterheld, M., Broccoli, V., Acampora, D., Simeone, A. and Wurst, W. (2003). Location and size of dopaminergic and serotonergic cell populations are controlled by the position of the midbrain-hindbrain organizer. *J Neurosci* 23, 4199-207.

Prakash, N., Brodski, C., Naserke, T., Puellas, E., Gogoi, R., Hall, A., Panhuysen, M., Echevarria, D., Sussel, L., Weisenhorn, D. M. V., Echevarria, D., Sussel, L., Weisenhorn, D. M., Martinez, S., Arenas, E., Simeone, A., Wurst, W. (2006). A Wnt1-regulated genetic network controls the identity and fate of midbrain-dopaminergic progenitors *in vivo*. *Development* 133, 89-98.

Stem cell-based strategies for the treatment of Parkinson's disease

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Parkinson's disease (PD) is characterized by the loss of dopaminergic (DA) neurons in the substantia nigra. The current therapy is based on the administration of L-DOPA, a precursor of dopamine. However the course of the disease is not altered by that treatment and as the cell loss progresses, L-DOPA loses effect. Transplantation of human foetal tissue containing mesencephalic DA neurons is an experimental therapy that has obtained promising results in PD patients, but the difficulty in obtaining sufficient tissue for transplantation has limited its application. Current therapeutic strategies aim at either stimulating endogenous neural stem cells to give rise to DA neurons or at transplanting stem cells engineered to differentiate into DA neurons.

With regard to the engineering of stem cells for transplantation, our work has focused on understanding midbrain DA neuron development with the idea of identifying new molecular players that could contribute to improve the differentiation of stem cells into DA neurons. We previously reported that expression of Nurr1 in neural stem cells (NSCs) was necessary but not sufficient to promote DA differentiation. We found that non-autonomous signals derived from glial cells were also required for DA differentiation of NSCs. More recently we found that Wnts are some of the signals regulating diverse aspects of midbrain DA neuron development. These factors are currently being applied to different types of stem cells and tested in animal models of PD. We think that such factors may contribute to improve cell replacement strategies for PD.

Adult nigral neurogenesis in Parkinson's Disease

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The recent discovery that the adult mammalian brain has the potential to generate new neurons and to integrate them into existing circuits has caused a shift in our understanding of how the central nervous system functions in health and disease. It has been consistently demonstrated, in two distinct areas of the forebrain, that mature cells in all neural lineages, including neurons, are generated throughout adulthood. Neuroblasts born in the adult subventricular zone (SVZ) subadjacent to the ependyma lining the lateral ventricles migrate along the rostral migratory stream to the olfactory bulb where they become interneurons. Neuroblasts born in the adult subgranular zone (SGZ) of the dentate gyrus migrate into the adjacent granular layer where they become granular neurons. Parkinson's disease (PD) has received much attention in recent years with regard to adult neurogenesis, since the degenerative process is relatively selective for the dopaminergic nigrostriatal projection. We have evaluated the published evidence that (1) dopamine plays a role in the regulation of constitutive neurogenesis in the adult brain and that (2) adult neurogenesis can repair the damaged nigrostriatal dopaminergic system.

In the last two years a large amount of converging data have consistently shown that the neurotransmitter dopamine stimulates endogenous adult neurogenesis in the SVZ by activating D2-like receptors on transit-amplifying progenitor cells. It remains to be studied in detail, however, whether decreased neurogenesis in diseases, such as PD, that are characterized by dopamine depletion, has clinically relevant functional consequences. Nevertheless, since precursor cells in the adult mammalian brain are pharmacologically accessible to systemic administration of dopamimetic drugs, stimulation of endogenous neurogenesis appears to be a potential strategy for a cell replacement therapy of the brain in diseases, in which SVZ-derived cells appear to contribute to repair processes. In contrast, the existence of neurogenesis *in vivo* in the adult mammalian substantia nigra remains controversial, although progenitor cells that can differentiate into neurons are present. The species of rodent studied, the manner in which BrdU was administered, or the delay between mitotic labelling and analysis might explain some discrepancies. Other confounding factors are summarized in Table 1. Because of the chronic progression of neuronal cell loss in PD, the therapeutic interest of stimulating adult neurogenesis in the substantia nigra is great, but the genesis *in vivo* of dopaminergic neurons in the substantia nigra from precursor cells in the adult mammalian brain has yet to be unequivocally demonstrated, dampening for the present our enthusiasm for this therapeutic approach.

In vitro differentiation of human umbilical cord blood progenitor cells

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Human Cord Blood (HCB) is considered a new valuable pool in stem cell research. HCB contains in its mononuclear cell fraction a certain number of mesenchymal stem cells (MSC) that are able to proliferate and to differentiate *in vitro* into many different cell types. MSC derived from HCB have the same potential as the MSC derived from bone marrow. Our objective for this project is to propagate HCB derived MSC and to study their differentiation development *in vitro*.

HCB probes are collected after informed consent of mothers according to the German guidelines for blood donation. Mononuclear cells were isolated from HCB by Ficoll density-gradient centrifugation and cultured in MSC medium. The MSC are growing as adherent cell fraction and they are cultured until their morphology changes from flattened to spindle-shaped. In this stage, the cells are pre-differentiated with bFGF and EGF containing medium for one week. Pre-differentiated cells are plated on fibronectin coated cover slips and cultured in different media for further differentiation. Cells are fixed at several differentiation time points (24 hours up to 16 days) and processed for immunocytochemistry against different markers.

The different HCB samples exhibit a wide range in number as well as in survival pattern of mononuclear cells. After three weeks in MSC culture medium the cells reach the stage of morphology of spindle-shaped and start proliferating faster whereas some probes keep growing in flattened morphology. At this stage, bFGF and EGF exposure changes their morphology towards neuronal like phenotype. Our results show that it is possible to isolate MSC out of HCB, proliferate and differentiate them to cells expressing stem cell markers as well as neuronal markers. A detailed analysis of the differentiation potential of HCB derived MSC will be presented at the meeting.

Transcription profiling of adult and fetal human neural progenitor cells

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Global gene expression profiling was performed on adult human hippocampus-derived neuroprogenitor cells (NPCs), fetal human frontal NPCs, adult human mesenchymal stem cells (MSCs) as a multipotent adult stem cell control, and adult human hippocampal tissue as a differentiated adult neural tissue control, to identify genes whose change in expression correlate with loss of multi-lineage potency and the begin of the differentiation process. The results were compared with data from various databases. Hierarchical cluster analysis of all neuroectodermal cell/tissue types revealed strong relationship of adult hippocampal NPCs with various white matter tissues, while fetal NPCs strongly correlate with fetal brain tissue. However, adult and fetal NPCs share the expression of a variety of genes known to be related to signal transduction, cell metabolism and neuroectodermal tissue. In contrast, adult NPCs and hMSCs both express genes mainly involved in extracellular matrix. We present for the first time a detailed transcriptome analysis of human adult NPCs suggesting a relationship between hippocampal NPCs and white matter-derived precursor cells. We further provide a framework for standardized comparative gene expression analysis of human adult brain-derived NPCs with other stem cell populations or differentiated tissues.

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Epigenetic modification in self-renewal and differentiation of neural stem cells

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The mechanisms underlying the regulation of neural stem cell (NSC) self renewal and differentiation are slowly being revealed. In both processes, transcription of the proper gene programs has to be activated or repressed. Important mechanisms in transcription regulation are the modulation of chromatin by DNA (de)methylation and by histone (de)acetylation. Self-renewal of stem cells in general implies repression of genes that encode for cell lineage differentiation. Enhancer of zeste homolog 2 (Ezh2) is a Polycomb group protein (PcG) with methyltransferase activity involved in stem cell renewal and maintenance by inducing gene silencing via histone methylation and subsequent histone deacetylation. To establish the possible role of Ezh2 in the maintenance and differentiation of NSCs, we have examined the expression of Ezh2 in NSCs isolated from embryonic (E14) and adult mice during proliferation and differentiation *in-vitro*. In addition, we have investigated the involvement of histone acetylation in NSC differentiation using the HDAC inhibitor trichostatin A (TSA).

Our results showed that Ezh2 is highly expressed in proliferating (undifferentiated) NSCs. In accordance with its suggested role, the expression of Ezh2 decreased when the NSCs differentiated into neurons and was completely inhibited during differentiation into astrocytes. Surprisingly, Ezh2 remained (over)-expressed in NSCs that differentiated into oligodendrocyte precursors. We found that hyperacetylation of NSCs by TSA appeared to significantly promote their differentiation into functional mature cholinergic neurons with extensive dendritic trees while inhibiting astrocyte differentiation.

Our study shows that further elucidation of the chromatin modulation mechanisms may provide ways to manipulate NSC proliferation and differentiation *in-vitro*, of importance to obtain large amounts of specific differentiated neural cell types for cell replacement transplantation purposes.

Long-term in vitro transduction of human neural stem cells

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Human neural stem cells (hNSCs) are currently under investigation for applications in cell-replacement therapies and basic neuroscience research. In the present study, we developed a hNSC-specific model for the long-term transduction of disease-related genes in vitro. hNSCs were transduced by using two different strategies: the non-viral nucleofection-technology of AMAXA and viral infection with the helper-dependent canine adenovirus (CAV-2) vectors. Additionally, we analysed the reporter gene expression of EGFP with two different promoter systems, the cytomegalovirus (CMV)-promoter and the cellular-virus hybrid chicken-beta-actin(CBA)/CMV-promoter, for efficient transgene expression. The highest transduction efficiency was obtained by nucleofection of CBA-promoter driven gene expression with $57\% \pm 9\%$. In the presence of drug selection the transduction efficiency increased up to $67\% \pm 11\%$. After 6 weeks in culture the first colonies of stable transduced hNSCs were formed with CBA-promoter driven constructs. In contrast, no stable transduced hNSCs were detected at that point of time with CMV-promoter driven constructs. Stable transduced cells were analysed by fluorescence microscopy, immunocytochemistry and flow cytometry. Stable transduced cells expanded over a period of 6 month and still had the potential to differentiate into neurons and glial cells.

Taken together, we developed a model for long-term transgene expression in human neural stem cells in vitro. The genetically modified hNSCs still showed crucial characteristics of neural stem cells including self-renewal and differentiation potential for up to 6 month. Furthermore, long-term, stable transduction of hNSCs with the fluorescent protein EGFP offers a reliable gene marking procedure allows following transplanted cells in cell-replacement therapies.

Fri, 01.12.06

ABSTRACTS VI

Human Embryonic Stem Cells as Donor Source for Neural Transplants

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Human embryonic stem cells (hESC) are expected to have far reaching applications in regenerative medicine. In principle, they represent a donor source for cell replacement in a large variety of tissues, including the nervous system. An intriguing question is whether these pluripotent cells can be used as a source of somatic stem cells for tissues with limited regenerative potential and whether such in vitro generated somatic stem cells display intrinsic and physiological properties comparable to primary cells. We have generated stably proliferating neuroepithelial stem cells from human ES cells (hES-NSCs). These cells can be extensively proliferated, show multipotentiality at a clonal level and maintain a constant neuro- and gliogenic differentiation pattern. In vitro, they give rise to functional neurons of mostly inhibitory phenotypes. RT-PCR and immunofluorescence data indicate that spontaneously differentiating hES-NSCs exhibit a regionally restricted identity compatible with a ventral and anterior hindbrain fate. Remarkably, even after long-term propagation, hES-NSCs remain responsive to instructive regionalization cues. Exposure of hES-NSCs to Shh and FGF8 induces ventral midbrain markers and enables the derivation of large numbers of TH-positive neurons. Retinoic acid treatment promotes further posteriorization with expression of caudal Hox genes. The neurogenic potential of hES-NSCs is conserved in vivo. Four months after transplantation into newborn SCID beige mice, hES-NSCs-derived neurons can be detected in various brain regions where they functionally integrate and exhibit spontaneous postsynaptic currents. Thus, stably proliferating hES-NSCs may provide a useful tool for studying mechanisms of human neural lineage segregation and a donor source for nervous system repair.

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Requirements for cell sorting and cell type analysis of stem cell-derived neural cell suspensions for transplantation

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Clinical neurotransplantation studies using fetal midbrain tissue have shown that nigral (A9) dopamine (DA) neurons can alleviate symptoms in patients with Parkinson's disease (PD). Derivation of this specific cell subtype from human embryonic stem cells (hESC) *in vitro* and its usage *in vivo* are complicated by the presence of unwanted cell populations such as immature stem cells and other neural and non-neural subsets.

Specific labeling of cell types by either promoter-driven fluorescence or by surface antigens, in combination with cell sorting methodologies for neuronal cells, enable the selection, detailed analysis and application of better-defined neural cell suspensions, which may be critical for establishing future hESC-based therapies of PD.

**Neural induction of human ES cells:
An overview and new directions**

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Human embryonic stem cells (hESCs) can be coaxed into specific developmental lineages and hold great promise as an unlimited source for cell therapy and drug screening. Since pluripotent hESCs mimic aspects of early development, these cells represent a powerful and unique model system to study human embryology, so far not accessible for experimentation. A better understanding of early developmental events and cellular fate choice are key issues for controlled manipulation and large-scale directed differentiation of hESCs. This talk will discuss the classical developmental concept of the "default mechanism" of neural induction (studied almost exclusively on animal but not human cells) in the context of hESC differentiation. Based on this, the current situation and rationale of commonly used empirical techniques (e.g. embryoid bodies, neural rosette formation) to convert hESCs into a neural fate will be reviewed and recent progress in the field highlighted.

Stem cell tracking in *in vivo* animal models using Magnetic Resonance Imaging: Perspectives and challenges.

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Magnetic Resonance Imaging (MRI) offers the possibility to monitor implanted cells *in vivo*. This is usually performed after cell labelling with iron oxide based contrast agents. Visualisation of small cell cluster *in vivo* and single cells in phantom experiments is possible. A number of different labelling strategies exist ranging from pinocytosis, microinjection, electroporation to lipofection.

In order to avoid possible misinterpretation due to other sources of hypointensity (dark contrast), the use of lanthanide chelates as MRI contrast agents was explored to generate a pronounced hyperintense (bright) contrast. Although lanthanide chelates have yet been used in only a few feasibility studies for the detection of cell deposits, they have a highly interesting potential as a tool to obtain not only information about the location but also about the cellular functional status.

Aspects involved in cell labelling, its evaluation *in vitro* and in animal models will be discussed. Labelling strategies will be discussed, which i) show an efficient incorporation of label into the cells, ii) are well tolerated by the cells, and iii) generate a sufficiently strong T1 contrast in MR images. This strategy is evaluated in different cell lines for its general applicability. In a second phase, *in vivo* detectability of such labelled cells after implantation into the host is assessed.

An advantage of lanthanide chelates is the possibility to use them as base for so called responsive contrast agents that are only activated as a function of enzyme activity / gene expression in the cell. First proof-of-principle experiments for this class of MRI contrast agents will be presented.

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ABSTRACTS VII

Neural stem cells and stroke

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Stroke is a common neurodegenerative disorder and one of the leading causes of death and disability in adult humans. In ischemic stroke, occlusion of a cerebral artery leads to focal ischemia in a restricted CNS region and death of different types of neurons and glial cells. Treatments to support efficient functional recovery in stroke patients are lacking. Cells from different sources have been tested for their ability to reconstruct the forebrain and improve function after transplantation in animals subjected to stroke. The transplanted cells can survive and partly reverse some behavioral deficits. However, the underlying mechanisms are unclear and there is little evidence for neuronal replacement. Recent findings in rodents that stroke leads to increased, long-term generation of neurons from neural stem cells (NSCs) in the subventricular zone, suggest an alternative approach to cell therapy in stroke based on self-repair. The newly formed immature neurons migrate into the damaged area, where they express markers of those mature neurons which died due to ischemic insult. Whether the new neurons formed after stroke are functional is unknown. However, even re-establishment of only a fraction of damaged neuronal circuitries could have significant implications. The optimum strategy for functional recovery after stroke would probably be to combine transplantation of NSCs with stimulation of neurogenesis from endogenous NSCs. However, before clinical trials are initiated, we need to know much more how to control stem cell proliferation and differentiation into specific phenotypes, induce their integration into existing neural and synaptic circuits, and optimize the functional recovery in animal models closely resembling the human disease.

Neural progenitors as alternative cell source combined with neurotrophic factors

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Primary dopaminergic (DA) neurons, which have so far been used as cell replacement therapy in Parkinson's disease, show several limitations including limited supply of donor tissue, poor graft survival and difficulties to clearly identify transplanted cells *in situ*. In addition, graft survival and clinical outcome have been very variable and ethical issues remain. Therefore, alternative cell sources for transplantation strategies have to be developed [1]. Generation of DA neurons from neural progenitors [2] or embryonic stem cells seem to be promising and could offer a better defined and standardized cell source for clinical transplantation.

We expanded E12 derived neural stem cells up to 40 fold and subsequently differentiated them into DA neurons. These cells were characterized using morphological, biochemical, molecular biological and electrophysiological techniques both, *in vitro* and *in situ* and exhibited all DA characteristics. In addition, behavioural tests revealed functional improvements similar to E14 derived cells after transplantation [3]. However, after transplantation the gain in number was diminished.

To overcome this limitation, we try to combine the stem cell approach with neurotrophic factors. The latter ones (e.g. GDNF, BDNF, FGF) have already been applied using different protocols like viral gene transfer [4] or co-transplantation [5]. FGF-2, one of the most potent mitogenes, promotes DA neuron survival both, *in vitro* and *in vivo* [5]. Interestingly, we found that FGF-2 deficient mice have less DA neurons after development compared to wild-type mice and that its receptor FGFR3 plays a crucial role for DA neuron development.

Recently, we transfected progenitor cells with reporter genes and FGF-2 using different non-viral methods [6]. The transfected cells still differentiated into the desired phenotype and survived transplantation. At present we try to immortalize the cells and transfect the cells with different growth factors in order to compare them.

- [1] Bjorklund, A. *Cell therapy for Parkinson's disease: problems and prospects. Novartis Found Symp.* 265:174-87; 2005.
- [2] Studer, L.; Tabar, V.; McKay, R.D. *Transplantation of expanded mesencephalic precursors leads to recovery in parkinsonian rats. Nat Neurosci.* 1(4):290-5; 1998.
- [3] Timmer, M.; Grosskreutz, J.; Schlesinger, F.; Krampfl, K.; Wesemann, M.; Just, L.; Bufler, J.; Grothe, C. *Dopaminergic properties and function after grafting of attached neural precursor cultures. Neurobiol Dis.* 21(3):587-606; 2006.
- [4] Kirik, D.; Rosenblad, C.; Bjorklund, A.; Mandel, R.J. *Long-term rAAV-mediated gene transfer of GDNF in the rat Parkinson's model: intrastriatal but not intranigral transduction promotes functional regeneration in the lesioned nigrostriatal system. J Neurosci.* 15; 20(12):4686-700; 2000.
- [5] Timmer, M.; Muller-Ostermeyer, F.; Kloth, V.; Winkler, C.; Grothe, C.; Nikkhah, G. *Enhanced survival, reinnervation, and functional recovery of intrastriatal dopamine grafts co-transplanted with Schwann cells overexpressing high molecular weight FGF-2 isoforms. Exp Neurol.* 187(1):118-36; 2004.
- [6] Cesnulevicius, K.; Timmer, M.; Wesemann, M.; Thomas, T.; Barkhausen, T.; Grothe, C. *Nucleofection is the most efficient non-viral transfection method for neuronal stem cells derived from ventral mesencephali with no changes in cell composition or dopaminergic fate. Stem Cells.* 10; 2006.

Neurotrophic factor therapy in Alzheimer's disease

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Over the last 2 decades numerous animal studies have demonstrated that nerve growth factor (NGF) can prevent lesion-induced and age-related degeneration of cholinergic neurons in the basal forebrain of rodents and primates. Although rodent and primate studies confirmed that NGF infusions are highly effective in preventing age-related and lesion-induced degeneration of cholinergic neurons in the basal forebrain, clinical translation of these findings was initially hampered by the inability to deliver NGF in a localized manner without adverse effects on non-targeted structures in the peripheral and central nervous system. Extensive studies demonstrating efficacy and safety of localized, cellular NGF delivery in rodent and primate models have led to the first gene therapy trial in Alzheimer's disease using autologous fibroblasts genetically modified to express NGF. Subjects (n=8) diagnosed with mild AD received cellular grafts of NGF expressing cells to the nucleus basalis in a phase I dose escalation study. Two sedated but non-anesthetized subjects moved as cells were intracerebrally injected, causing bleeds; subsequent injections performed under anesthesia were without complication. After follow-up up to 5 years, no adverse events related to NGF delivery have been observed. Cognitive testing of subjects at different time points post-surgery suggested an improvement in the rate of cognitive decline, and PET scans showed a significant increase in cortical activity. Brain autopsy from one subject demonstrated robust neurite growth responses to NGF. Therefore, additional studies of NGF gene therapy in patients with Alzheimer's disease are warranted.

Animal studies conducted with adeno-associated virus and lentiviral vectors support the fact that in vivo NGF gene delivery is equally effective in preventing the degeneration of cholinergic neurons. As in vivo gene delivery is much simpler than the implantation of autologous, genetically modified cells, current studies focus on AAV-mediated NGF delivery, and a phase I trial of AAV mediated NGF delivery is currently underway. The transition of the growth factor field to novel delivery methods that achieve adequate doses of growth factors in the CNS, while preventing growth factor spread to non-targeted regions, may provide the first real opportunity to test whether these neuroprotective proteins can impact human neurological disease.

In addition to NGF, other neurotrophic factors such as BDNF might be able to prevent or delay degeneration of other neuronal populations such as cortical neurons, severely affected in AD. Combinations of trophic factors with other disease-modifying therapies, such as anti-amyloid approaches, could optimize eventual treatments of AD.

News on Multiple System Atrophy (MSA)

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MSA frequently presents with levodopa unresponsive Parkinsonism reflecting degenerative loss of striatal dopamine receptors. The primary objective of neuroregenerative therapy in MSA therefore is to provide dopamine receptors using striatal cell grafts. In previous pilot studies we have been able to demonstrate regeneration of dopaminergic responsiveness in unilateral double lesion rat models of striatonigral degeneration, the neuropathological substrate underlying Parkinsonism in MSA. More recently we have assessed the effects of primordial striatal grafts on impaired dopaminergic sensitivity of spontaneous motor deficits. In addition, we have studied the integration of primordial striatal grafts in a novel transgenic mouse model of MSA that replicates both oligodendroglial alpha-synuclein inclusions and MSA like neuronal pathology. Further studies will be required to establish the striatal lesion as key target of neuroregeneration for MSA associated Parkinsonism. In parallel, a number of phase II trials involving growth hormone and minocycline have established the European MSA Study Group as a functioning network that will facilitate the clinical application of neural transplantation in MSA.

Long-term expanded neurospheres fail to survive following intracerebral transplantation

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As we have previously shown, with continued time in culture there is a marked decrease in the ability of neurospheres derived from the fetal murine or human brain to form viable grafts after transplantation into the adult and neonatal rat brain.

We have now carried out a time-course study, looking at human neurospheres following 2, 8, 14 and 20 weeks in culture and comparing the viability of grafts derived from these spheres 4, 8, 12 and 24 weeks after transplantation into neonates. The neonatal model was chosen for its ability to provide the cells with a supportive environment in which cues for neuronal development are still present, and in which there is no problem with rejection.

After only two weeks in culture, neurospheres formed large grafts, which generally survived well until at least 12 weeks following transplantation. With continued expansion, however, spheres formed smaller grafts, until at 20 weeks in culture, no surviving grafts were seen, even at very early time points.

To determine whether this is due to a dilution of true neural stem cells with time in culture, we derived pure neural stem cells from the 8 week expanded neurospheres following an established protocol (Conti et al 2005). These cells homogeneously expressed the neural progenitor markers sox-2, 3CB2 and nestin and were expanded as NS cells for 5 months before transplantation into the neonatal rat brain. However, as with long-term expanded neurospheres, these cells showed limited survival in the host brain.

Conti, L. et al. Niche-independent symmetrical self-renewal of a mammalian tissue stem cell. *PLOS Biol.* 9 (3): 1594-1606; 2005

The biochemistry of neuromelanin granules

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Neuromelanin (NM) granules are organelles underlying the characteristic pigmentation of several catecholaminergic neurons in the substantia nigra (SN) and the locus coeruleus of primates. There is increasing evidence that NM granules are generated by a genetic program to exert specific functions in these brain areas and the function of iron storage is severely impaired, e.g., in Parkinson's disease (PD). In PD NM granules significantly accumulate iron, which is detectable via transcranial ultrasonography and may represent a biomarker for PD progression. Additionally, α -synuclein, the key protein of Lewy bodies, deposits onto NM granules in PD, but not under physiological condition. *In vitro*, isolated NM decreases the activity of the proteasome, induces mitochondrial oxidative stress, and thereby may contribute to neurodegeneration and PD progression.

Despite the fundamental role of NM granules, knowledge about their molecular composition was lacking due to the general absence of a functional model system. To characterise NM granules and to clarify their origin we thus applied subcellular proteomics following organelle isolation from the human SN.

Our results provide the first biochemical description of human NM granules. We identified 72 proteins, which are mainly found in lysosomes and in lysosome-related organelles (LRO's), in particular enzymes involved in macromolecule degradation, traffic, prevention of oxidative stress or pigmentation. Nevertheless, some proteins are not found in lysosomes, but in LRO's. LRO's, e.g. osteoclast granules, platelet dense granules or melanosomes, are constituents of highly specialized cell types and exhibit functions different from lysosomal macromolecule turnover, such as bone remodelling, blood clotting or pigmentation.

For the first time we uncovered the biogenesis of NM granules to originate from the endosomal system. Furthermore, these data strongly support the hypothesis of a physiological role of NM granules in the human brain that gets severely altered in PD.

Neuronal differentiation of mesenchymal stem cells depends on PKA-pathway and on intracellular Ca²⁺-concentration

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In the present work, we aimed to verify the potential of MSC's to differentiate into neurons. The transdifferentiation of mesenchymal stem cells (MSC) into neurons is still a matter of controversy, and the mechanisms involved in this process remain largely unknown. The derivation of mature neurons from MSC was described only in co-culture systems, what raises the question if the differentiation is a potential of the cells itself, or if it was possible only due to fusion with mature neurons.

MSC were isolated from 20 healthy human subjects and characterized by FACS-analysis. Multipotentiality was addressed by differentiating them into chondrocytes and osteocytes. Afterwards, cells were differentiated in a medium containing either cAMP, or IBMX, or a combination of both, or no cytokine. Different calcium-blockers or PKA-inhibitor were used in order to impair differentiation, which was quantified with NF-200 immunostaining. Patch-clamp recording was used to confirm neuronal phenotype. Pictures were taken in confocal microscope and for statistical analysis we used ANOVA.

The isolated cells expressed CD90, 105, 44, and 13, and were negative for CD34 and 45. After isolation, we were able to differentiate them into chondrocytes and osteocytes. In the control situation, no NF200 expression was seen. On the other hand, 10.75%±1.35 (p<.0001) of positivity was seen under IBMX, or 15.18%±1.12 in a combination of cAMP and IBMX (p<.0001). Na⁺ and K⁺-voltage gated currents were recorded. Differentiation was impaired with PKAi (5.73%±0.42, p<.0001), nifedipin (5.79%±0.98, p<.0001), Ni (7.06%±1.68, p<.0001), or Cd (0±0, p<.0001).

We were able to isolate a population of stromal stem cells from the bone marrow of human subjects, since they were multipotential and self-renewable. The increase of the concentration of cAMP raised the percentage of neurons up to 15%. Neuronal differentiation could be observed without fusion with other cell types, and it seems to be dependent on the PKA pathway, but also involved the intracellular concentration of Ca²⁺.

Autologous adult neural progenitor cell transplantation represents a feasible strategy to promote structural repair in the chronically injured spinal cord

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Adult neural progenitor cells (NPC) represent an attractive source for cell-based regenerative strategies in central nervous system (CNS) disease. In animal models of spinal cord injury, syngenic adult NPC, which were isolated from pooled post-mortem CNS tissue and co-transplanted together with fibroblasts, have been shown to promote substantial structural repair. The autologous transplantation of adult NPC represents a major advantage compared to other sources of neural stem/progenitor cells. However, the feasibility of autologous NPC generation from a single biopsy in a relevant preclinical CNS disease model has yet to be demonstrated. To investigate this matter, adult Wistar rats underwent a cervical spinal cord lesion followed 2 days later by a minimal subventricular zone aspiration biopsy. NPC were isolated and propagated separately for each animal for the following 8 weeks. Thereafter, they were co-transplanted with simultaneously harvested skin fibroblasts in an autologous fashion into the cervical spinal cord lesion site. Four weeks later, graft survival, tissue replacement and axonal regeneration were assessed histologically. Animals receiving either allogenic NPC combined with fibroblasts or autologous pure fibroblast grafts served as control groups. Within 8 weeks after the biopsy more than 3 million NPC could be generated from a single aspiration biopsy, which displayed a differentiation pattern undistinguishable from syngenic NPC grafts. NPC within autologous co-grafts readily survived, replaced cystic lesion defects completely and differentiated exclusively into glial phenotypes, thus paralleling previous findings with syngenic NPC. The delayed transplantation 8 weeks after the spinal cord lesion elicited substantial axonal regeneration. These findings demonstrate that the therapeutic strategy to induce structural repair by transplanting adult autologous NPC after the successful propagation from a small brain biopsy into an acute CNS disease model such as spinal cord injury is feasible on the preclinical level.

Uracil nucleotides stimulate human neural precursor cell proliferation and dopaminergic differentiation

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Neural stem/precursor cells (NPCs) serve as a model of brain development, providing promise for currently incurable brain diseases and injuries. Nucleotides and their receptors are present very early during development and may influence proliferation and differentiation of various precursor cells. In human midbrain derived neural precursor cells we detected functional P2 receptors via calcium imaging and identified them as P2Y₁, P2Y₄, P2Y₆ and P2X₄ receptors via RNA analysis. UTP a P2Y_{2/4} agonist stimulated proliferation (1 μM) which was abrogated by the P2Y receptor blocker PPADS. Treatment with UTP (100 μM) during differentiation increased the number of TH-positive cells and TH protein by 267% and 319%, respectively. UTP-stimulated dopaminergic differentiation was blocked by P2 receptor antagonists suramin (10 μM) or PPADS (100 μM). Treatment of cells with 1 μM and 10 μM UDP during the differentiation period, increased TH expression in immunoblots to 194 ± 7% (n = 3) and 180 ± 8%, respectively. During differentiation, treatment with UTP stimulated the extracellular signal-regulated kinase (ERK) pathway. Both, ERK1/2 phosphorylation and dopaminergic differentiation were reverted by U0126, a selective ERK kinase (MEK) inhibitor, as well as by suramin. When other P2 receptor agonists (ATP, ADP and ADPβS, all 100 μM) were applied, both proliferation and dopaminergic differentiation of hNPCs were compromised. Our data suggest that uracil nucleotides can be employed to enhance, proliferation and/or dopaminergic differentiation of human NPCs, possibly via P2Y₄ and P2Y₆ receptor activation.

Effects of fibroblast growth factor (FGF)-20 on the differentiation of human embryonic stem cells into dopaminergic neurons

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Fibroblast growth factor (FGF)-20 is interesting in the context of dopaminergic (DA) neurons for several reasons. In the adult rat midbrain, FGF-20 and its receptor FGF receptor 1 (FGFR-1) are preferentially expressed in DA neurons. In vitro, FGF-20 exerts a trophic effect on cultured rat midbrain DA neurons and induces a neuron-like morphology as well as tyrosine hydroxylase (TH) expression in neural stem cells overexpressing Nurr1. Moreover, certain Fgf20 gene haplotypes are associated with the increased risk of Parkinson's disease. We have analyzed the effects of FGF-20 upon the differentiation of human embryonic stem cells (hESCs, SA002 cell line) into DA neurons. Co-culturing the hESCs with PA6 mouse stromal cells induced neuronal differentiation. The addition of FGF-20 (1 ng/ml) to the medium further promoted neuronal differentiation. In the presence of FGF-20 the number of nestin-positive cells increased 2-fold and the number of bIII-tubulin-positive cells by a factor of 4. Furthermore, 9% of the hESC-derived cells were immunopositive for TH in the presence of FGF-20, while without FGF-20 supplementation the proportion of TH-positive cells was 4%. Semi-quantitative RT-PCR confirmed that FGF-20 promotes differentiation of hESCs into DA neurons, as evidenced by higher expression of Th, Aldh1a1, Pitx2 and En1 genes. Taken together, our data suggest that addition of FGF-20 to the culture medium can increase the yield of DA neurons from hESCs grown on top of PA6 stromal cells.

The survival signaling kinase AKT/PKB induces trophic effects in murine models of Parkinson's disease

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In spite of promising preclinical studies, neurotrophic factors have not yet achieved an established role in the treatment of human neurodegenerative diseases. One impediment has been the difficulty in providing these macromolecules in sufficient quantity and duration at affected sites. An alternative approach is to directly activate, by viral vector transduction, intracellular kinase signaling pathways that mediate neurotrophic effects. We have therefore examined whether AKT (protein kinase B) mediates trophic effects on normal adult, neurotoxin-lesioned and aged dopamine (DA) neurons of the mouse substantia nigra (SN) by AAV transduction with a constitutively active form.

A myristoylated, constitutively active form of AKT (Myr-AKT-FLAG), was packaged into an adeno-associated-viral (AAV1) transfer vector. Either AAV-Myr-AKT or AAV-GFP (vector control) was stereotaxically injected into the left SN. In a neurotoxin model of Parkinson's disease (PD), mice were injected with 6-Hydroxydopamine into the left striatum three weeks later. AAV-Myr-AKT provided almost complete protection from SN DA neuron loss at 28 days post-lesion. In addition, whereas many prior attempts to block neuronal apoptosis have offered cell body protection in the absence of axon fiber preservation, AAV-Myr-AKT also provided preservation of striatal DA fibers as measured by the optical density. The functional significance of this protection was confirmed by preservation of striatal DA levels and improved apomorphine-induced rotational behavior in the AAV-Myr-AKT injected animals.

In addition, AAV-Myr-AKT had pronounced trophic effects on DA neurons of normal adult and aged mice, including increases in neuron size, phenotypic markers and sprouting, associated with a functional behavioral correlate.

These studies support the feasibility of direct activation of intracellular survival signaling pathways by viral vector transduction in the treatment of PD.

Loss of dopaminergic responsivity in the double lesion SND/MSA-P rat model

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The parkinsonian variant of Multiple System Atrophy (MSA-P) is characterized by a loss of dopaminergic neurons comparable to that found in Parkinson's disease. In addition, progressive loss of striatopallidal projections is thought to account for L-Dopa unresponsiveness in MSA-P. Whereas histological features of MSA-P have been successfully reproduced in the double lesion SND/MSA-P rat model, loss of L-dopa responsivity has so far not been demonstrated in this model.

15 male Wistar rats received a stereotaxic unilateral 6-hydroxydopamine (6-OHDA) injection into the left medial forebrain bundle, followed by two stereotaxic injections of 75 nmol quinolinic acid (QA) each into the ipsilateral striatum. Forelimb akinesia was assessed by the stepping and the cylinder test without drugs and following dopaminergic stimulation with L-Dopa (8mg/kg i.p.). For lesion assessment, sections were stained with TH and DARPP-32.

The marked contralateral forelimb stepping deficit observed following 6-OHDA improved significantly after challenge with L-Dopa ($p < 0.001$). This responsivity to L-Dopa was abolished by the subsequent striatal QA lesion. In the cylinder test, the marked asymmetry observed after unilateral 6-OHDA lesioning was reversed by L-Dopa to baseline levels. Following QA lesion animals almost exclusively used the ipsilateral paw, with cylinder test performance under L-Dopa failing to reach baseline ($p = 0.001$) or 6-OHDA + L-Dopa ($p = 0.002$) levels. Nigral cell loss ($90\% \pm 5\%$) correlated with both stepping deficit ($r = 0.561$, $p = 0.015$) and responsivity to L-Dopa ($r = 0.608$, $p = 0.008$) as well as cylinder test results ($r = 0.656$, $p = 0.005$). Lesion size of the dorsal striatum correlated significantly with the loss of L-Dopa response ($r = 0.593$, $p = 0.01$) in the stepping test.

These findings contribute further to the behavioral characterization of the double lesion rat model of MSA, improving its value in the evaluation of future neurorestorative strategies.

A mechanism for the proliferative action of dopamine in the SVZ

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Cambridge Centre for Brain Repair, CB2 2PY, United Kingdom

The discovery of stem cells has brought about the possibility of using cell-based therapies to treat a wide range of injuries and diseases in humans. The occurrence of neural stem cells (NSCs) in the adult central nervous system (CNS), that under certain conditions are able to proliferate and renew neuronal numbers, has raised great expectations for replacing the dopaminergic cells lost in Parkinson's Disease (PD).

Indeed, it is potentially possible to harness this capacity either indirectly (first *in vitro* expansion before their transplantation), or directly (increase of local proliferation), to facilitate the generation of specific cell types in order to replace missing neurons in neurodegenerative diseases. Elucidating the mechanisms involved in the latter is the main focus of my work.

Dopamine has previously been shown to enhance the proliferation of endogenous NSCs lining the lateral walls of the lateral ventricle, by stimulation of dopamine receptors expressed by these same cells. We have also replicated this effect *in vitro* in our laboratory, through the use of neural precursor cells, derived from adult subventricular zone and E16 mouse striatum, both of which were expanded in culture as neurospheres for seven days. We have recently shown that growth factors are implicated in this mechanism. We are currently investigating the effect of dopaminergic agonists on growth factors present in the SVZ and the mechanism through which the proliferative effect of dopamine is accomplished. In doing so, a potential pharmacological method for replacing the specific degenerated cells lost in PD seems possible.

General Information

General Information

Venue Information

The meeting will be held in the "Runder Saal" of the Konzert Haus Freiburg, Konrad-Adenauer-Platz 1, 79098 Freiburg, Germany, email: konzerthaus@fwtm.freiburg.de, website: www.konzerthaus.freiburg.de.

The conference venue can only be reached through the Dorint Hotel directly next to the Concert House. Please follow on-site signs.

The Concert House can be reached from the EuroAirport Basel, Mulhouse, Freiburg by taxi shuttle services and bus transfer. Information can be found under www.euroairport.com. The conference venue can also be reached by train www.db.de which is only 2min walk on foot to the Concert House.

Hotel Information

Dorint Hotel

Konrad Adenauer Platz 2
79098 FREIBURG
GERMANY

Tel : (+49)761/38890

Fax : (+49)761/3889100

E-mail : h5383@accor.com

URL:

http://www.accorhotels.com/accorhotels/fichehotel/gb/nov/5383/fiche_hotel.shtml

The meeting discount price per night is EUR 115 for a either single or double room including breakfast buffet and tax. Hotel accommodation in the Dorint-Hotel Freiburg should be directly booked via the hotel reservation office.

For further accommodation options in Freiburg please turn to the tourist information website of the City of Freiburg www.freiburg.de.

Registration Desk

A registration desk will be open in front of the "Runder Saal" in the Concert House during the following times:

Thursday, 30.11.06 12:00h-19:00h

Friday, 01.12.06 08:00h-19:00h

Matters concerning hotel accommodation at the Dorint Hotel should be dealt with at the hotel's registration desk individually.

Manuela Fellbach will be at the registration desk for all queries and additionally all members of the local support team can be approached throughout the conference with all questions to the logistics and organization.

Instructions to speakers

The 17th NECTAR meeting will have a dense and intense schedule as always. Therefore it is *important that speakers present their talks within the allotted time* (25min for guest speakers and 8min for each datablitz presenter, including discussion time). Please bring your talks on USB-sticks or CD to the media desk in the "Runder Saal". Standard powerpoint projection facilities will be available and technical assistance for your presentation will be there throughout the conference.

Internet webpage

NECTAR:

www.nectar-org.eu

University Hospital Freiburg:

www.uniklinik-freiburg.de

Laboratory of Molecular Neurosurgery, Freiburg:

www.nikkhah-lab.org

General Information

Arrangement

The meeting fee of EUR 250 per participant and EUR 150 for students covers attendance at the conference, drinks during breaks, and lunches on Friday and Saturday, as well as the meeting dinner on Friday evening at the "Historische Kaufhaus". Your name badge serves as ticket.

Disclaimer

The local and scientific organizing committees accept no liability for injuries and losses of whatever nature incurred by participants and/or accompanying persons, nor loss of, or damage to, their luggage and/or personal belongings.

Meeting dinner at the Historisches Kaufhaus

The Friday evening dinner and concert will take place at the "Historische Kaufhaus", a building situated directly at the famous Freiburg Münster. It is a 10min walk on foot from the Concert House to the dinner venue taking you through the old historical centre of Freiburg:

Historisches Kaufhaus
Münsterplatz 24
79098 Freiburg

Tourist Information Freiburg / Cultural highlights in Freiburg during NECTAR 2006

For further information concerning Freiburg and accommodation, restaurants, bars and places to see, please refer to www.freiburg.de.

Furthermore if you seek some non-science entertainment during the NECTAR meeting there are the following cultural highlights to see in Freiburg and surrounding cities:

Thursday, 30.11.06

- Konzerthaus Freiburg: 20:00h

Classical Concert:

A Schönberg: "Verklärte Nacht", op. 4, P Tschaikowsky: Sextett op. 70 d-Moll
"Souvenir de Florence"

Freiburger Barockorchester, Leitung: Gottfried von der Goltz

- Historix Tour: 19:00h, Meeting point: Predigertor

"Bürger, Galgen und Alte Häuser"

On-foot tour through Freiburg at night- in German but very recommendable!

- Theatre at the Stadttheater Freiburg:

Großes Haus 19:30h

"Les Larmes du ciel- Die Tränen des Himmels"

Kleines Haus 20:00h

„Die Möglichkeiten einer Insel“

General Information

During the whole conference the following events take place in Freiburg:

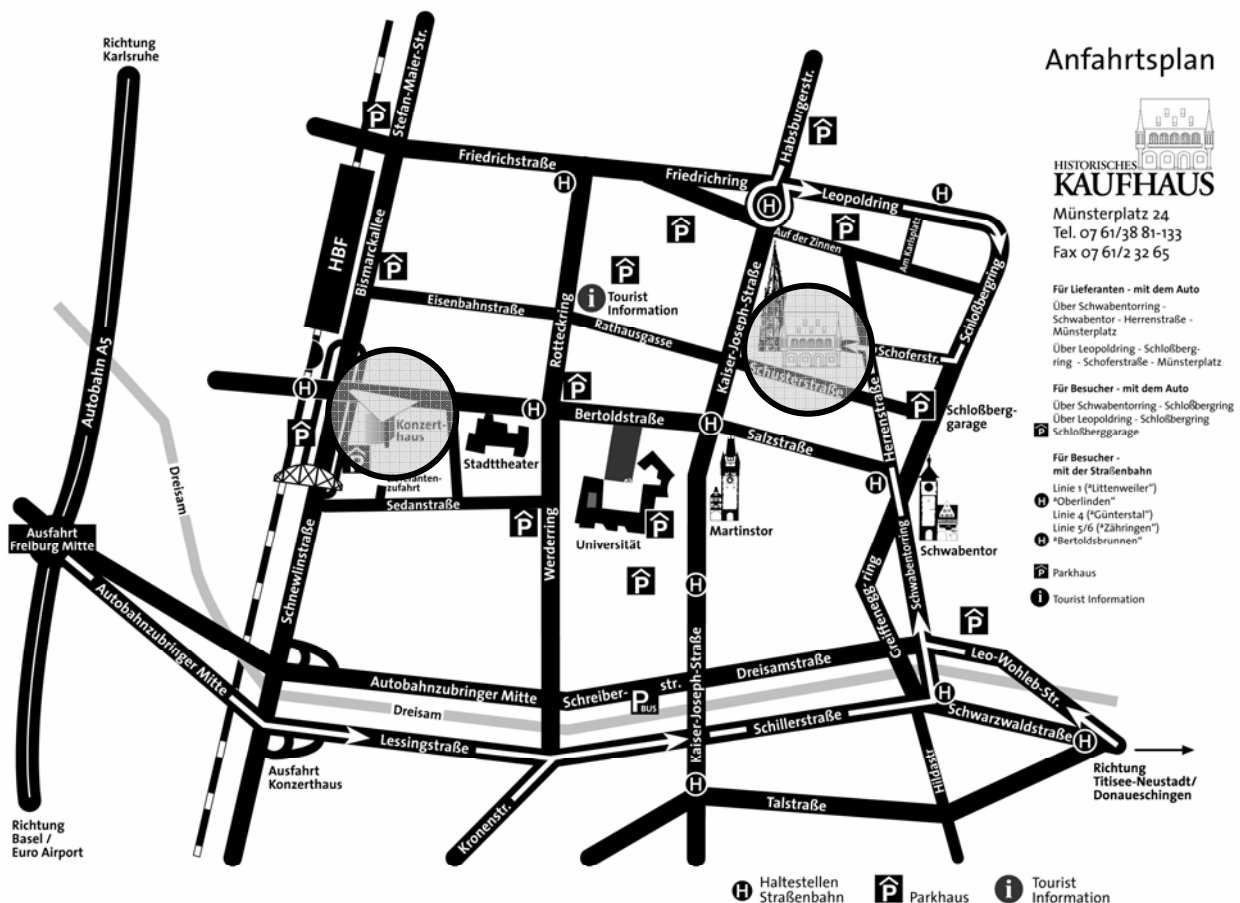
- Weihnachtsmarkt Freiburg:

Christmas market in the historical centre of Freiburg at the Rathaus-Platz
 Open 10:00h-20:30h Mo-Sat, Sunday open 11:30h-19:30h
www.weihnachtsmarkt-freiburg.de

- Palazzo Colombino:

Daily at 19:30h
www.palazzo-colombino-freiburg.de
 Combines an exclusive dinner created by renowned chefs and spectacular cabaret entertainment

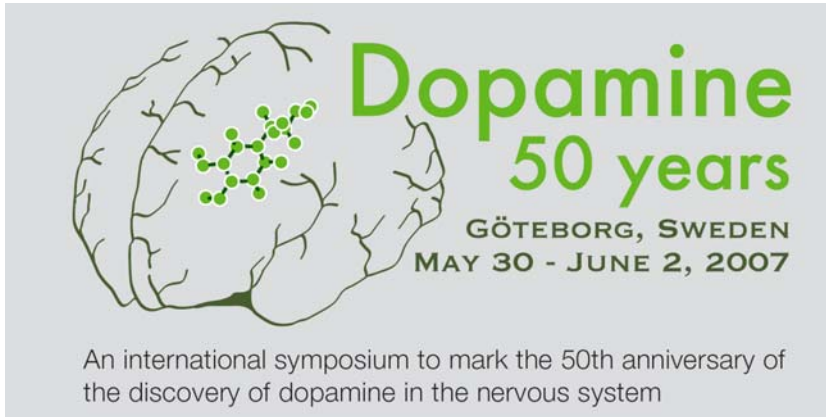
Map of the city and venues



General Information

Announcements

-



**Dopamine
50 years**
GÖTEBORG, SWEDEN
MAY 30 - JUNE 2, 2007

An international symposium to mark the 50th anniversary of the discovery of dopamine in the nervous system

The logo features a stylized brain with a cluster of green dots representing dopamine molecules. The text is in a green, sans-serif font.

- **NECTAR Meeting 2007**

06.-08.Dec.2007
Lund, Sweden



- **INTR-10 2008**

International Conference on Neural Transplantation and Repair

10-13.Sept.2008
Freiburg, Germany

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