

# **ABSTRACTS**



**THURSDAY 26<sup>TH</sup> NOVEMBER AM**

**SESSION 1**

**STEM-HD**

**Session 1**

**STEM-HD**

08.30-08.35	Marc Peschanski	Welcome
08.35-09.00	Beatrice de Schepper	The European Huntington's Association
09.00-09.30	Marc Peschanski	Stem cells for therapeutics and exploration of mechanisms in Huntington's disease
09.30-10.00	Elena Cattaneo	Cholesterol defect in HD
10.00-10.30	Nick Allen	Neural differentiation of human embryonic stem cells: applications to STEM-HD
<b>10.30-11.0</b>	<b>Coffee Break</b>	
11.00-11.30	Noel Buckley	Transcriptional programming in HD
11.30-12.00	Anne Rosser	Developing therapies for Huntington's Disease
12.00-12.30	Gilles Bonvento	Mechanisms of striatal neurprotection by CNTF: A potential therapy for HD
<b>12.30-13.30</b>	<b>Lunch</b>	



## THE EUROPEAN HUNTINGTON'S ASSOCIATION

Beatrice de Schepper

### STEM CELLS FOR THERAPEUTICS AND EXPLORATION OF MECHANISMS IN HUNTINGTON'S DISEASE

Marc Peschanski, on behalf of the partners of the STEM-HD European Network

INSERM/UEVE U861, I-STEM, AFM, Evry France”

Embryonic stem cell (ES) lines have two fundamental attributes: they may be expanded indefinitely at an undifferentiated state, allowing for production of massive numbers of genetically specified cells, and they can be triggered to differentiate into any cell type, allowing for analysis of a specific population characterized by stage and type of differentiation. *STEM-HD* aimed at making use of these two attributes to explore the mechanisms of Huntington's disease (HD), using human ES cell lines derived from embryos identified as expressing the mutant HD gene during a pre-implantation genetic diagnosis procedure. Using these cell lines, the consortium did:

- Establish protocols to enrich ES cell progeny in phenotypes of interest. HD effects primarily a mature cell population of medium-spiny GABAergic neurons in the striatum; these were obtained using pharmacological induction and selection in *in vitro* culture;
- Design and implement infrastructures for mass cell production and long-term cultures of ES cells either undifferentiated or following guided differentiation, in order to exploit fully the intrinsic capacities of the ES cell line in the production of massive numbers of specifically differentiated cells;
- Identify, using differential transcriptomics for the full human genome, “biomarkers” associated to the mutant gene in the HD-expressing ES cells progeny. These are used as test signals for subsequent analyses although association of these molecular markers to the disease has not been validated using experimental cell models and patients' samples, yet;
- Explore the mechanisms of the disease in the mutant HD-expressing ES cells progeny to determine proteins involved in signalling pathways attached to the HD mutation;
- Perform high content drug screening on HD gene-carrying ES cell progeny.

## CHOLESTEROL DEFECT IN HUNTINGTON'S DISEASE

Elena Cattaneo

### NEURAL DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS: APPLICATIONS TO STEM-HD

**Nick Allen**<sup>1</sup>, Alysia Battersby<sup>1</sup>, Amanda Redfern<sup>1</sup>, Peter Giles<sup>1</sup>, Lesley Jones<sup>1</sup>, Andrew Hollins<sup>1</sup>, Shona Joy<sup>1</sup>, Rickie Patini<sup>2</sup>, Siddharthan Chandran<sup>2</sup>, Maxime Feyeux<sup>3</sup>, Nathalie Lefort<sup>3</sup>, Laetitia Aubry<sup>3</sup>, Aurore Bugi<sup>3</sup>, Alexandra Benchoua<sup>3</sup>, Philippe Tropel<sup>3,4</sup>, Stéphane Viville<sup>4</sup>, Claudia Spits<sup>5</sup>, Karen Sermon<sup>5</sup>, Marc Peschanski<sup>3</sup>, Anselme L. Perrier<sup>3</sup>

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In vitro disease modelling is emerging as one of the major applications of human stem cell technologies. The rationale for the STEM-HD programme is that human ES cell lines derived from HD genotyped blastocysts provide a resource for human neural cell derivation for in vitro studies and drug screening. To address the different cell requirements of the programme robust differentiation protocols have been developed. Firstly, antagonism of TGFbeta signalling in defined media conditions is used to derive neural progenitor cells that can be expanded over several passages to provide uniform populations for screening purposes. Secondly, directed differentiation protocols have been developed that sequentially promote forebrain and then ventral forebrain specification of progenitors to derive cells neurons that express markers of striatal medium spiny neurons.

Available HD human ES cell lines carry mutant Htt alleles with 40-45 expanded CAG repeats. To begin to screen for HD associated phenotypes and identify potential biomarkers relevant to presymptomatic HD we have first obtained gene expression profiles from ES cells and neural progenitor cells from three HD ES cell lines (SI-187, VUB05 and Huez2.3) and three wild type cell lines (SA01, VUB01 and H9) using Illumina Human WG-6 v3 expression beadchips. The preliminary transcriptomic analysis will be presented and a future perspective will be discussed.

## **TRANSCRIPTIONAL PROGRAMMING IN HD**

**Noel Buckley**

## **DEVELOPING THERAPIES FOR HUNTINGTON'S DISEASE**

**Anne E Rosser**

Cardiff University

Although there has been growing international interest in identifying new treatment strategies for Huntington's disease, treatments currently available for clinical use are extremely limited and are related to symptom relief rather than disease modification. To date, many new substances tested in clinical studies have been used on the basis of their potential neuroprotective properties or on the basis of their efficacy in other neurodegenerative diseases. However, it is likely that the most effective treatments for Huntington's disease will grow out of a detailed understanding of the pathogenic processes, and that progress in this area will depend on the generation of better animal and cellular models of the disease

## **MECHANISMS OF STRIATAL NEUROPROTECTION BY CNTF, A POTENTIAL THERAPY FOR HUNTINGTON'S DISEASE**

**Gilles Bonvento**

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Ciliary neurotrophic factor (CNTF) is a neurotrophic cytokine released by astrocytes that stimulates the survival of developing neurons. CNTF is also neuroprotective in various models of acute neuronal death and has been proposed as a neuroprotective agent for Huntington's disease (HD). CNTF administration in the striatum protects medium spiny neurons against Quinolinic acid (QA) in rodents and primates. A phase I clinical trial confirmed the safety of local brain administration of encapsulated cells genetically engineered to produce CNTF and reported a recovery of somatosensory evoked potentials in patient implanted with capsules releasing the largest amount of CNTF. Despite these encouraging results, the mechanisms mediating CNTF neuroprotective effect are still unclear. The change in astrocyte phenotype triggered by CNTF in the adult brain suggests that this cytokine may have an indirect neuroprotective effect through activated astrocytes. Indeed, we have recently shown that CNTF-activated astrocytes display marked phenotypic and molecular changes associated with an improved handling of extracellular glutamate in the rat striatum. We suggested that such effect could be mediated by an increased function of astrocyte glutamate transporters (GTs), GLAST and GLT-1. These two GTs uptake the bulk of extracellular glutamate and this function is crucial to prevent accumulation of glutamate to excitotoxic levels. Using lentivirus-mediated CNTF overexpression in the rat striatum, whole-cell patch-clamp and extracellular electrophysiological recordings on corticostriatal slices, we provide evidence that CNTF neuroprotective effects against QA are mediated through an enhanced glutamate uptake by activated astrocytes.

**THURSDAY 26<sup>TH</sup> NOVEMBER PM**

**SESSION 2**

**STEM CELLS**

**Session 2**

**Stem cells**

13.30-13.40	Christian Winkler	President of NECTAR opening remarks
13.40-14.25	Ian Wilmut	iPS technology in research and repair
14.25-14.50	Anselme Perrier	Towards allograft transplantation in <i>Macaca fascicularis</i> model of Huntington's disease: Production of striatal graft from monkey embryonic stem cells
14.50-15.20	Datablitz 1	
<b>15.20-15.40</b>	<b>Coffee Break</b>	
15.40-16.05	Siddharthan Chandran	An overview of oligodendrocytes from ES cells
16.05-16.35	Lorraine Iacovitti	The current status of directed stem cell differentiation into dopaminergic neurons for transplantation in Parkinson's disease
16.35-17.00	Paul De Sousa	The road to delivery of human embryo stem cell derived therapies
17.00-17.30	Pete Coffey	Stemming vision loss using stem cells - seeing is believing
<b>17.30-17.40</b>	<b>Break</b>	
17.40-18.25	Peter Harper	Huntington's Disease; some historical insights

**Datablitz 1:**

- 1 HUMAN UMBILICAL CORD BLOOD-DERIVATIVES FOR TREATMENT OF GLOBAL CEREBRAL ISCHEMIC INJURY IN ONE YEAR OLD CHILD – A CASE STUDY

**Mirosław Janowski**

- 2 HUMAN NEUROEPITHELIAL STEM CELL

**Anna Falk**

- 3 A MULTIPLEXED PROTEOMICS APPROACH TO IDENTIFYING NOVEL PROTEINS DIRECTING DOPAMINERGIC NEUROGENESIS IN THE DEVELOPING VENTRAL MIDBRAIN

**Rowan Orme**

- 4 PHYSIOLOGICAL LEVEL OF OXYGEN PROMOTES MATURATION OF NEURONS FROM MOUSE EMBRYONIC STEM CELLS AND REMOVES TUMORIGENIC CELLS

**Eunju Jenny Shin**

- 5 FROM HUMAN ES CELL TO EXPANDABLE MULTIPOTENT NEURAL STEM CELL

**Lucy A. Crompton**

- 6 VIRUS-FREE GENERATION OF INDUCED PLURIPOTENT STEM CELLS FROM PRIMARY HUMAN FETAL FIBROBLAST CULTURES

**P. Capetian**

## **IPS TECHNOLOGY IN RESEARCH AND REPAIR**

### **Ian Wilmut**

MRC Centre for Regenerative Medicine, University of Edinburgh, 49, Little France Crescent, Edinburgh EH16 4SB,

Stem cells hold great promise for the development of new treatments for human disease. While most public attention is paid to the use of cells for transplantation to replace those that are damaged or lost as a result of disease this is to overlook the potential use of cells in research, drug discovery and toxicology.

In particular the opportunity to have in the laboratory the tissues that are affected in an inherited disease and to compare these with tissues from a healthy donor will provide new insight into the molecular cause of the disease. It may then be possible to devise high-through put assays able to identify small molecules that are able to prevent the development of disease symptoms. Work is in progress to study inherited forms of ALS. In addition, human cells in the laboratory may provide important new approaches to the safety testing of new drugs. Extensive tests are required for new drugs developed for treatment of any disease. Despite this effort many compounds are withdrawn late in the assessment process because they cause unacceptable side effects in some patients. This late withdrawal of new compounds wastes the very large sums that have already been spent in development and initial screening of new compounds and contributes a significant proportion of the total costs of drug development. . Human cells derived from stem cells may offer opportunities to identify and eliminate compounds with unacceptable side effect at an earlier stage in the process. Liver, heart and nerve cells would be particularly useful for this purpose.

**TOWARDS ALLOGRAFT TRANSPLANTATION IN *MACACA FASCICULARIS* MODEL OF HUNTINGTON'S DISEASE:  
PRODUCTION OF STRIATAL GRAFT FROM MONKEY EMBRYONIC  
STEM CELLS**

**Anselme L Perrier**

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Huntington's disease (HD) is characterized by striatal atrophy associated with massive neuronal loss of the GABA-ergic medium spiny projection neurons of the caudate and putamen (MSN). MSN are DARPP32+, a potent protein phosphatase-1 regulated by both dopaminergic and glutamatergic receptor. DARPP32+ neurons constitute over 90% of the entire neuronal population of the striatum. HD is partially amenable to treatment by substitutive (foetal) cell therapy. However, this technique is marred by logistic problems that restrict considerably the number of patients who may benefit from it. Potent alternative sources of cells are therefore acutely needed. Due to their original properties, human embryonic stem cells (hESC) are prime candidates.

We have demonstrated (Aubry et al., PNAS 2008) that hESC differentiation can be directed into striatal neurons both *in vitro* and *in vivo* after transplantation and thus suggested the possible therapeutic potential of hESC for HD. However, the therapeutic assessment of human striatal graft, now used in HD clinical trial across Europe, was successfully tested only in primate models of HD mostly because: (1) human graft mature too slowly to allow functional assessment in rat, (2) behavioural and cognitive tests are more relevant in primate than in rat, (3) allograft of monkey striatal cells in monkey mimics best "clinical transplantation" with regard to immune response of the host to the graft.

Consistently, we have adapted our striatal differentiation protocol to monkey-ESC in order to produce *in vitro* striatal graft. Here we present the differentiation of ORMES-18 monkey ES cells into striatal progenitors and neurons FOXG1+/DARPP32+ both *in vitro* and *in vivo* after transplantation in rat model of HD. Assessment over 4 months of the proliferation and the neuronal commitment properties of these culture showed that monkey cells expanded much less considerably in the host brain than human cells and did generate numerous monkey post-mitotic striatal neurons (DARPP32+). These results confirmed the feasibility of the assessment of ES-derived graft for HD in monkey models in allograft conditions.

## HUMAN UMBILICAL CORD BLOOD-DERIVATIVES FOR TREATMENT OF GLOBAL CEREBRAL ISCHEMIC INJURY IN ONE YEAR OLD CHILD – A CASE STUDY

Mirosław Janowski<sup>1</sup>, Tomasz Kmiec<sup>2</sup>, Elzbieta Jurkiewicz<sup>3</sup>, Tomasz Kropiwnicki<sup>4</sup>, Aleksandra Habich<sup>1</sup>, Katarzyna Kotulska<sup>2</sup>, Elzbieta Jelonek<sup>5</sup>, Anna Sarnowska<sup>1</sup>, Mieczysław Litwin<sup>6</sup>, Dariusz Boruczkowski<sup>7</sup>, Barbara Lukomska<sup>1</sup>, Jerzy Walecki<sup>8</sup>, Marcin Roszkowski<sup>4</sup>, Sergiusz Józwiak<sup>2</sup>, Krystyna Domańska-Janik<sup>1</sup>

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<sup>7</sup>Polish Stem Cell Bank, Warsaw

<sup>8</sup>Department of Diagnostic Radiology, Central Hospital of the Ministry of Interior and Administration in Warsaw

**Background:** Basing on the recent experimental results Human Umbilical Cord Blood (HUCB) derivatives transplantation can be proposed as a new treatment for stroke. In the first clinical approach described herein we administered an autologous, neurally committed HUCB-progenitor cells (PC) directly into cerebral ventricular system of the global cerebral ischemia patient.

Case history: One year old child experienced devastating, cardiac arrest-induced cerebral ischemia with a diagnose of vegetative state three months thereafter. Following next three months the child received monthly 3 subsequent intraventricular infusions of HUCB-PCs. Transplanted cells were prelabeled with SPIO for MR monitoring. Now the patient, who was firstly transplanted in July 2008, has finished the twelve month period of follow up and underwent all required examinations and tests previously approved by the ethical commission of The Children's Memorial Health Institute in Warsaw, Poland. The employed method for cells transplantation and monitoring was found to be safe and efficient for acquiring MR signal from the cell cluster ( $>10^6$  cells) most probably formed in the wall of a lateral ventricle at the side of the first cell injection. No adverse events or abnormal reaction to implantation, except of transient increases in body temperature, was then observed. Mild functional improvement was found – decreased nystagmus, spasticity and the number of epileptic seizures. The features of the child contact with parents has appeared. Thus vegetative state can not be diagnosed at this time. However it is difficult to judge either central delivery of HUCB-PCs or spontaneous recovery was decisive for the observed mild clinical improvement.

**Conclusions:** This first case have demonstrated that intraventricular HUCB-PC transplantation is safe and can be monitored by MRI. The further studies would clarify the benefits and any detriments to this approach.

## HUMAN NEUROEPITHELIAL STEM CELL

**Anna Falk** and Austin Smith

Wellcome Trust Centre for Stem Cell Research, University of Cambridge

### **Background**

Human neural stem (NS) cell lines have the potential to provide a renewable source of neurons for basic and applied research, pharmaceutical screening, and possibly cell replacement therapy. We have recently derived tripotent human NS cells from human foetal brain and characterised them carefully (Sun et al., *Molecular and Cellular Neuroscience*, 2008, 38:245-258; Sun et al., *PLOS One*, 2009;4:e5498 ). We have now successfully derived neural stem cell lines from both human ES cells and human iPS cells. These cells are distinct from the foetal NS cells and show features of more primitive neuroepithelial cells. We have therefore named them neuroepithelial-like stem (NES) cells.

### **Results**

NES cells are capable of differentiation into neurons, astrocytes and oligodendrocytes in vitro. Significantly, NES cells appear to be responsive to inductive cues to generate a wider range of neuronal subtypes than the human foetal NS cells which seem restricted to production of Gabaergic neurons. The NES cells grow as a monolayer with a doubling time of 24 hours in the presence of EGF and FGF2 in a flower-like organisation. They are highly neurogenic, producing around 70% neurons after four weeks of differentiation in vitro. NES cell lines can be expanded massively in culture, over 100 passages, while maintaining a normal karyotype and without losing their differentiation potential.

### **Conclusion**

Human NES cell lines represent a novel cell type with a potential to provide a renewable source of neurons for basic and applied research, pharmaceutical screening, and possibly cell replacement therapy.

## A MULTIPLEXED PROTEOMICS APPROACH TO IDENTIFYING NOVEL PROTEINS DIRECTING DOPAMINERGIC NEUROGENESIS IN THE DEVELOPING VENTRAL MIDBRAIN

Rowan Orme, Monte Gates and Rosemary Fricker-Gates.

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**Background:** Stem cell-derived dopaminergic neurons are seen as a future source of cells for transplantation in PD. Current differentiation protocols do not produce homogenous DA neuron populations required for safe cell therapy. We hypothesise that further, as yet undetermined, proteins are required to fully differentiate stem cells into homogenous DA neuron populations.

**Aims:** By performing a multiplexed, quantitative proteomic investigation into the developing midbrain of rats before (E11), during (E12) and after (E13, E14) peak midbrain DA neurogenesis, we aim to discover novel signalling proteins critical for the specification of DA neurons. Protein expression patterns between the four ages of tissue will be compared using isobaric tagging for relative and absolute quantitation (iTRAQ) reagents.

**Methods:** Proteomic analysis was used to study proteins directly, rather than infer their presence as with microarray technology. Proteins were extracted from the ventral midbrain of rats aged E11 to E14, digested with trypsin and labelled using iTRAQ reagents. Liquid chromatography and tandem mass spectrometry were used to identify proteins and compare protein expression across the four tissue ages.

**Results:** Over 3000 proteins expressed in the developing VM around the time of peak dopamine neurogenesis have been identified and their expression profiles quantified. Many proteins that would be expected to be identified were observed, including mature neuron (e.g. Tuj-1) and dopamine markers (e.g. TH). Importantly, several known DA determinants were identified (e.g. Foxa1), suggesting the technique is capable of identifying novel signalling factors. Western blotting and immunolabelling of cryostat sections confirmed protein expression data predicted by iTRAQ analysis.

**Conclusions:** The method described permits identification of novel signalling proteins from the developing VM. These could advance current differentiation protocols for the production of transplantable mDA neurons.

## PHYSIOLOGICAL LEVEL OF OXYGEN PROMOTES MATURATION OF NEURONS FROM MOUSE EMBRYONIC STEM CELLS AND REMOVES TUMORIGENIC CELLS

Eunju Jenny Shin<sup>1,2</sup>, Nicholas R. Forsyth<sup>1</sup>, and Rosemary A. Fricker-Gates<sup>1</sup>

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**Background:** Embryonic stem cells (ESCs) have a great potential to generate any kinds of cells in the body which will be very useful for cell therapy such as Parkinson's and Huntington's disease. Establishing the culture environment as similar as physiological condition would help maintaining and differentiating ESCs. One of the important conditions is the level of oxygen. Conventionally 20% room oxygen level has been used to culture cells but it is in fact much higher level than the physiologic oxygen level (2%). Studies reported that ESCs in physiologic oxygen showed enhanced ESC clonal recovery and decreased chromosomal abnormalities.

**Aims:** In this study, we used mouse ESC line 46C to see the effect of physiologic oxygen on neuronal differentiation and proliferation during the monolayer differentiation protocol.

**Methods:** mESCs were cultured in high (20%) and low (2%) oxygen level for 5 days before differentiation and introduced to differentiation protocol to both conditions from each condition (H-H, H-L, L-H, L-L). During the differentiation period, some sets of cells were treated with puromycin to select Sox1<sup>+</sup> neural precursors. Cells were fixed and immunostained with Oct4, Ki67, GFP,  $\beta$ -III-tubulin, and GABA antibodies.

**Results:** mESCs in low oxygen level during the differentiation showed less proliferation (Ki67<sup>+</sup> cells) and more importantly complete lose of tumorigenic population (Oct4<sup>+</sup> cells). Neural precursors expressing a higher level of GFP was reduced in low oxygen conditions. The percentage of neurons generated from both conditions was not significantly different but neurons generated from low level of oxygen showed more mature morphology with an increased number of primary neurites and increased levels of GABA neurotransmitter.

**Conclusions:** It might be necessary to culture mESCs in low oxygen conditions to differentiate them to become GABAergic neurons to be used in cell transplantation, both to eliminate the Oct4<sup>+</sup> dividing stem cells and for better differentiation.

## FROM HUMAN ES CELL TO EXPANDABLE MULTIPOTENT NEURAL STEM CELL

**Lucy A. Crompton**, Hannah Taylor, Kei O. Cho, Maeve A. Caldwell

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Neural differentiation of Human Embryonic Stem Cells (hESCs) provides an important model for early neural stem cell (NSC) development, as well as a potential source of transplantable material for therapeutic use in neurodegenerative disease. HESCs are advantageous to their fetal counterparts because they are essentially a 'blank-canvas' and can be programmed to become a NSC, with the potential to become any cell type within the brain. HESC derived NSCs demonstrate greater plasticity, resulting in enhanced regenerative properties, thus increasing the potential success rate of cell transplantation.

The hESC lines used in this study, Shef2 & -3, were two of the first lines to be distributed by the UK-SCB and are some of the most widely used lines in the UK. However, to date there is no available protocol for the production of NSCs from these lines. We have developed a 23-day 3-stage protocol, which reproducibly generated a pure population of NSCs, which were expandable in long-term culture. Upon *in vitro* differentiation, NSCs exhibited a primarily neurogenic fate, readily differentiating into dopaminergic neurons. Furthermore, calcium imaging of differentiated neurons revealed they possess electrical activity and are thus functional. Together these results suggest that our 3-stage protocol can differentiate hESCs into NSCs, which fulfil criteria for use a developmental model of neurogenesis, and potentially for *in vivo* cell replacement therapy.

## VIRUS-FREE GENERATION OF INDUCED PLURIPOTENT STEM CELLS FROM PRIMARY HUMAN FETAL FIBROBLAST CULTURES

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**Background:** The generation of induced pluripotent stem cells (iPS) from somatic cells opens up new perspectives for cell transplantation in and research on neurodegenerative diseases. While most protocols up until now employed retroviral transfection of somatic cells (mainly fibroblasts), the use of plasmids for reprogramming is much cheaper and easier to employ. A lately published protocol by Kenji et al. uses a single multiprotein expression vector, which comprises the coding sequences of c-Myc, Klf4, Oct4 and Sox2 linked with 2A peptides for the generation of iPS.

**Aims:** While the original protocol employed an immortalized human fetal lung fibroblast cell line, our group has been trying to reprogram primary fibroblast cultures from human fetuses after abortion.

**Methods:** An aborted fetus at gestational week 11 served after informed consent of the mother as donor for primary skin fibroblast cell culture. After one passage in culture, cells were transfected by electroporation with the 13 kB plasmid pCAG2LMKSimO (Kenji et al.) carrying the four Yamanaka factors and a reporter gene for mOrange. Expression of typical pluripotency markers (Oct 3, Nanog, Sox2, Rex1) has been studied by immuno-fluorescent stainings and RT-PCR. Further effort has been made to establish a pure iPS culture on different coatings (human fetal fibroblast feeders inactivated by Mitomycin C, Gelatin and Matrigel).

**Results:** After 8 days of culturing on gelatin coated dishes, colonies of cells expressing mOrange formed, some exhibiting the typical morphology of iPS/ES cells. The numbers of these cells increased over several passages. Further results of the characterization will be presented at the meeting.

**Conclusion:** We managed up until now to establish a self renewing cell line partly carrying the typical morphology of pluripotent cells.

## **AN OVERVIEW OF OLIGOENDROCYTES FROM ES CELLS**

**Siddharthan Chandran**

## **THE CURRENT STATUS OF DIRECTED STEM CELL DIFFERENTIATION INTO DOPAMINERGIC NEURONS FOR TRANSPLANTATION IN PARKINSON'S DISEASE**

**Lorraine Iacovitti**

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Parkinson 's disease (PD) is characterized by the progressive degeneration of dopamine (DA) neurons in the midbrain. Although the symptoms of early stage PD can be alleviated by a variety of palliative treatments, efficacy declines and treatment-related side effects emerge with the inexorable progression of the illness. Cell replacement, therefore, remains an important potential therapy, particularly at later stages of PD. The success of this approach, however, greatly depends on the discovery of an abundant source of cells which can *exclusively* develop into midbrain DA (mDA) neurons. Studies from our lab and others have shown that a subset of human neural progenitor cells (hNPs) derived from pluripotent human embryonic stem (hES) or induced pluripotent stem (hiPS) cells are *uniquely* able to differentiate into functional mDA neurons after transplantation into the PD rat striatum. These findings have led us to hypothesize that these cells are intrinsically fated to become mDA neurons. Supporting this contention, the mDA fate gene, LIM homeobox transcription factor 1 alpha (Lmx1a), has been shown to play a critical role in specifying their mDA phenotype. By tracking the expression of Lmx1a and other DA markers, like retinaldehyde dehydrogenase (Aldh1a1), TrkB receptor and tyrosine hydroxylase (TH), we have delineated individual lineage stages in the mDA fate restriction process as pluripotent cells transform into mDA-specified hNPs, mDA-specified precursors, and finally, differentiated mDA neurons. As not all hES or hiPS cells are mDA-specified, going forward, it will be important to develop a selection strategy for the sequestration of only those cells of appropriate stage and mDA specification for transplantation into the PD brain and to continue to search for factors capable of driving the mDA differentiation process.

## THE ROAD TO DELIVERY OF HUMAN EMBRYO STEM CELL DERIVED THERAPIES

**P.A. De Sousa**<sup>1,2</sup>,

University of Edinburgh<sup>1</sup>, Edinburgh, Scotland, UK; Roslin Cells Ltd<sup>2</sup>, Roslin Biocentre, Midlothian, Scotland, UK; Scottish National Blood Transfusion Service<sup>3</sup>, Edinburgh, Scotland, UK.

Realising the therapeutic promise of human embryo stem cells (hESCs) necessitates overcoming diverse challenges. This begins with meeting the legal and ethical challenges of procuring embryos. Next, the efficiency of *in vitro* culture systems supporting cells must be improved using reagents whose specification complies with regulatory standards. This is followed by qualification of resulting cells and assessment of their bio-safety both in relation to the prospective transplant recipient and the community at large. Complicating the address of these challenges are constantly evolving and internationally variant regulatory standards which have the capacity to negate the utility of cells for emerging therapies before clinical trials are even begun. To address these challenges Roslin Cells Ltd (RC) was established in 2006 as a not-for-profit company owned by the University of Edinburgh (UoE), the Scottish National Blood Transfusion Service (SNBTS) and the Roslin Institute. RC is licensed by the Human Fertilisation & Embryology Authority (HFEA) and Human Tissue Authority and is ISO9001 accredited. To date RC has isolated 7 new research grade hESCs and is currently actively producing clinical grade hESCs compliant with quality assured good manufacturing practice. These will be utilised in a Wellcome Trust funded consortium to produce clinical grade red blood cell concentrates led by the Scottish National Blood Transfusion Centre. While the manufacturing of regulator approved clinical grade hESCs compliant is now ongoing by ourselves and others internationally further research is required to evolve the chemical definition of culture environments which sustain these cells to obviate the potential for inadvertent pathogen transmission. Also important is a better understanding of the genetic stability of these cells and the susceptibility of these cells to infectious pathogens that may be introduced via primary tissue or exposure to crude or purified animal or human cell products. Collectively such knowledge will help ensure the biosafety of prospective hESC based therapies.

## **STEMMING VISION LOSS USING STEM CELLS - SEEING IS BELIEVING**

**Pete Coffey**

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The London Project to Cure Blindness aims to make the most of human embryonic stem cells to prevent blindness and restore sight in patients with Age-related Macular Degeneration (AMD) by 2012. Our goal is to replace cells essential for “seeing” lost by disease at the back of the eye. We aim to repair and regenerate the aged, diseased eye using human embryonic stem cells which have been transformed into the cells affected in AMD: the support cells for the photoreceptors (retinal pigment epithelium), and the photoreceptors. The cells will be surgically implanted into a clinical population of AMD patients.

## **HUNTINGTON'S DISEASE; SOME HISTORICAL INSIGHTS**

**Peter S. Harper,**

Institute of Medical Genetics, Cardiff University, Heath Park, Cardiff, CF14 4XN

Looking back at the history of HD shows how it has repeatedly shown us lessons which are applicable to much wider areas of medicine and science, and which are often as relevant today as when they first occurred. A few of these insights are discussed here; I hope that those working on HD today will continue to document more recent aspects that are already becoming part of history, so that others can learn from these in the future.



**FRIDAY 27<sup>TH</sup> NOVEMBER AM**

**SESSION 3**

**GENE THERAPY**

**Session 3**

**Gene Therapy**

08.30-08.55	Nicole Déglon	RNAi technology for the treatment of Huntington's disease
08.55-09.20	James Uney	Progress to date on therapeutic gene transfer in the CNS
09.20-09.45	Deniz Kirik	Gene therapy for dopamine replacement in Parkinson's disease
09.45-10.10	Bechir Jarraya	From Monkey brain to Patient brain: dopamine gene therapy for Parkinson's disease
<b>10.10-10.40</b>	<b>Coffee Break</b>	



## RNAI TECHNOLOGY FOR THE TREATMENT OF HUNTINGTON'S DISEASE

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Huntington's disease (HD) is a fatal autosomal dominant neurodegenerative disorder caused by a polyglutamine (polyQ) expansion in the huntingtin (htt) protein. No cure is available to date to alleviate neurodegeneration. Recent studies have demonstrated that RNAi represents a promising approach for the treatment of autosomal dominant disorders. We have demonstrated that nonallele-specific silencing of htt (siRNA targeting wild-type (WT) and mutant htt) is efficacious and well tolerated up to 9 months post-treatment (Drouet et al. 2009). However, the sihtt administration leads to transcriptomic changes with yet no identified functional consequences.

An allele-specific silencing would lead to the depletion of mutant huntingtin with preservation of its wild-type counterpart. We therefore recently designed shRNA targeting the disease isoform of heterozygous single-nucleotide polymorphism (SNP) present at high frequency in the human population. We showed that all siSNP efficiently degrade mutant htt mRNA and prevent the appearance of HD neuropathology *in vitro* and *in vivo*. On the contrary, the presence of one mismatch in the targeted mRNA prevented its degradation in almost all cases. These data constitute a proof of principle for allele-specific silencing of Htt and further support the therapeutic potential of RNAi for HD. However, the clinical implementation of such a strategy will require the development of several therapeutic products (siSNP) to treat a large proportion of HD patients.

**PROGRESS TO DATE ON THERAPEUTIC GENE TRANSFER IN THE CNS**

**James Uney**

## **GENE THERAPY FOR DOPAMINE REPLACEMENT IN PARKINSON'S DISEASE**

**Deniz Kirik**

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It has been hypothesized that complications related to oral administration of L-DOPA in Parkinson's disease (PD) could be due to the pulsatile supply of provided by peripheral administration. In small clinical trials testing continuous DA stimulation by long acting agonists or infusion of L-DOPA has been shown to reverse the side effects of intermittent treatment. Thus, we are currently developing a novel gene therapy strategy to provide long-term continuous DOPA administration using recombinant adeno-associated viral vector serotype 5 (rAAV5) to over-express tyrosine hydroxylase (TH) and GTP-cyclohydrolase (GCH1) enzymes. In previous work we have shown that the delivery of the two enzyme is sufficient to induce behavioral recovery and that the L-DOPA induced dyskinesias can be alleviated in rats rendered parkinsonian by a partial lesion removing about 75% of the nigrostriatal dopamine input. More recently, we determined the optimal stoichiometric relationship between TH and GCH1 genes for ectopic DOPA production and the cellular machinery involved in its synthesis, storage and metabolism. We found that the BH4 synthesis from ectopic GCH1 expression was saturable, yielding optimal TH enzyme functionality between GCH1:TH ratios of 1:3 and 1:7. When these vectors were injected at a GCH1:TH ratio of 1:5 into the striatum of rats with complete unilateral 6-OHDA lesion, the animals displayed near complete restoration in non drug-induced behavior tests, such as cylinder and stepping tests, after 12 weeks of transgene expression. Furthermore, we found that the continuous DOPA delivery protected the animals against developing dyskinesias after chronic daily subcutaneous L-DOPA injections. Taken together, these results strengthen the evidence that this gene therapy strategy provide robust means for continuous DA replacement that can be utilized as a competitive treatment alternative for PD patients, especially those in the complication phase.

## FROM MONKEY BRAIN TO PATIENT BRAIN : DOPAMINE GENE THERAPY FOR PARKINSON'S DISEASE

**Jarraya B**<sup>1,3</sup>, Ralph S<sup>2</sup>, Lepetit H<sup>3</sup>, Boulet S<sup>1</sup>, Jan C<sup>1</sup>, Bonvento G<sup>1</sup>, Azzouz M<sup>5</sup>, Miskin J<sup>2</sup>, Gurruchaga J-M<sup>3</sup>, Vinti M<sup>3</sup>, Fenelon G<sup>3</sup>, Brugiére P<sup>3</sup>, Kingsman S<sup>2</sup>, Hantraye P<sup>1</sup>, Remy P<sup>1,3</sup>, Mitrophanous K<sup>2</sup>, Mazarakis ND<sup>4</sup>, Palfi S<sup>1,3</sup>

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<sup>5</sup> Neurology unit, Medical school, Sheffield University, UK

Oral dopaminergic treatments have remained the primary standard of care for Parkinson's disease (PD) for the last 40 years. Although highly efficacious in the early stages of disease they are associated with debilitating long term side effects that seriously impact on the quality of life and restrict the longevity of such treatment. The severity of PD, lack of a cure and the limited long term effectiveness of current therapies allow for the consideration of novel therapeutic approaches.

We have developed a lentiviral vector (ProSavin®) derived from the equine infectious anaemia virus expressing the three key dopamine biosynthetic enzymes (tyrosine hydroxylase, aromatic L-amino acid decarboxylase and GTP cyclohydrolase-1). ProSavin was previously demonstrated to mediate dopamine production and cause behavioural correction in the 6-OHDA lesion rat model of PD (Azzouz et al., 2002). Further studies have demonstrated dopamine replacement and significant long term (>2 years) efficacy in a severe MPTP-lesioned non human primate model, following bilateral injection into the sensorimotor putamen. Following these proof of principle efficacy studies a series of preclinical studies has been performed showing that ProSavin is safe and well tolerated in toxicology studies.

A phase I/II clinical trial to evaluate the safety and efficacy in late stage PD patients has been approved and was initiated in December 2007 in France. The trial will evaluate the safety and efficacy profile of ProSavin following bilateral administration to the sensorimotor putamen.

A summary of the translation of the development of ProSavin from the bench to the clinic will be presented.

**FRIDAY 27<sup>TH</sup> NOVEMBER AM**

**SESSION 4**

**HUNTINGTON'S DISEASE**

**Session 4**

**Huntington's disease**

10.40-11.00 Datablitz 2

11.00-11.25 AC Bachoud-Lévi A preliminary report on outcomes in the French language multi-centre trials in Huntington's disease

11.25-11.50 Lesley Jones Genetic Modifiers for HD

11.50-12.15 Jan Vesper Pallidal Deep Brain stimulation (DBS) in Chorea Huntington

12.15-12.40 Máté Döbrössy Environmental and training associated factors in transplant-mediated brain repair: Does neurorehabilitation have a role in cell replacement therapy?

12.40-13.10 Datablitz 3

**13.10-14.00 Lunch**

**Datablitz 2:**

- 1 IS THERE A CORRELATION BETWEEN DONOR AGE AND EXPANSION OF TRANSPLANT VOLUME IN PATIENTS WITH HUNTINGTON'S DISEASE?

**Elisabeth Schültke**

- 2 RESTORATION OF SKILLED LIMB MOVEMENTS BY FETAL GABAERGIC GRAFTS IN A RAT MODEL OF HUNTINGTON'S DISEASE

**A. Klein**

- 3 DONOR AGE DEPENDENT GRAFT DEVELOPMENT AND RECOVERY IN A RAT MODEL OF HD; CELLULAR AND BEHAVIOURAL ANALYSIS

**Stefanie Schackel**

**Datablitz 3:**

- 1 THE PHARMACOLOGICAL MODULATION OF GRAFT INDUCED DYSKINESIA IN THE TRANSPLANTED HEMI-PARKINSONIAN RAT

**Gaynor Smith**

- 2 PREDICTIVE VALUE OF SEVERITY OF PREOPERATIVE L-DOPA-INDUCED DYSKINESIA FOR THE DEVELOPMENT OF GRAFT-INDUCED DYSKINESIA IN THE RAT PARKINSON MODEL

**Joanna García**

- 3 SEROTONIN NEURON-DEPENDENT AND -INDEPENDENT REDUCTION OF DYSKINESIA BY 5-HT<sub>1A</sub> AND 5-HT<sub>1B</sub> RECEPTOR AGONISTS IN THE RAT PARKINSON MODEL

**Manolo Carta**

- 4 HIGH SEROTONIN TRANSPORTER BINDING IN TWO PATIENTS WITH PARKINSON'S DISEASE AND STRIATAL FOETAL MESENCEPHALIC GRAFTS. IMPLICATIONS FOR GRAFT INDUCED DYSKINESIA?

**Marios Politi**

## IS THERE A CORRELATION BETWEEN DONOR AGE AND EXPANSION OF TRANSPLANT VOLUME IN PATIENTS WITH HUNTINGTON DISEASE?

**Elisabeth Schültke**, Irina Mader, Tobias Piroth, Michael Trippel, Jaroslaw Maciaczyk, Volkmar Glauche, Helga Schrecker, Bernhard Landwehrmeyer, Guido Nikkhah

Laboratory of Molecular Neurosurgery, Dept. of Stereotactic Neurosurgery, University Clinic Freiburg, Germany

**Background:** Between June 2005 and November 2008, a total of 20 patients with clinically and genetically verified Huntington's disease have been recruited in this study. Out of those, ten patients received transplants derived from the striata of 6 – 12 weeks old embryonic donors, while the other ten patients were followed as non-implanted controls. All transplanted patients received bilateral transplants into the caudate nucleus and the putamen. The transplantation procedure was conducted in two stages. Between 40 and 48 deposits of homogenized tissue were distributed between the target regions via 5 – 6 trajectories on each side.

**Aims:** The aim of this brief report is to discuss whether a correlation exists between the age of the donor, the alteration of the transplanted tissue volumes and the occurrence of graft-versus-host reactions.

**Methods:** Complete deformation based volumetry was performed in 3 patients (Dartel, Spin 8). Measurements were derived from pre-transplantation MRIs and post-transplantation MRIs obtained at 19 and 39 months after surgery.

**Results:** The preliminary analysis shows an expansion of the transplant volume in all analysed cases; a more detailed correlation with donor age will be presented at the meeting.

**Discussion:** It is not yet clear whether the volume increase is correlated with an improvement of function or simply a consequence of the transplantation procedure.

## RESTORATION OF SKILLED LIMB MOVEMENTS BY FETAL GABAERGIC GRAFTS IN A RAT MODEL OF HUNTINGTON'S DISEASE

Klein<sup>1</sup>; E.L. Lane<sup>2</sup>; S.B. Dunnett<sup>1</sup>

<sup>1</sup>Brain Repair Group, School of Biosciences, <sup>2</sup>Welsh School of Pharmacy; Cardiff University, Cardiff, Wales, United Kingdom

**BACKGROUND:** Skilled limb movements are severely impaired during the course of Huntington's disease (HD). Thus far, little attention has been paid to the qualitative and detailed analysis of reaching-for-food movements, i.e. skilled limb movements, in the quinolinic acid (QA) lesion rat model and the effects of whole ganglionic eminence (wGE) grafts.

**METHODS:** 21 Lister-Hooded rats were unilaterally infused with QA into the dorso-lateral striatum contralateral to their preferred paw. 11 rats served as non-operated controls. Six weeks after the lesion, 11 rats received 2µl of an E15 wGE cell suspension into their ipsilateral striatum (1 wGE/2µl). 10 rats were sham-transplanted. Before and after the lesion and following transplantation the rats' motor behaviour was analysed in the single pellet reaching test, the staircase test, the side-stepping/paw-placing paradigm, and the cylinder test. One week before perfusion the rats received a FluoroGold injection into the globus pallidus. The rats were transcardially perfused 19 weeks post transplantation.

**RESULTS:** The qualitative analysis of reaching behaviour revealed that GABAergic grafts had a strong beneficial effect on the rat's performance in the single pellet reaching test. In an overall analysis the transplanted rats performed nearly as well as the control rats. Interestingly, individual movement components responded differently to the grafts. Particularly distal rotatory limb movements were most impaired, but also well restored; reaching success of grafted rats nearly doubled compared to sham-transplanted rats, but still did not achieve performance equal to the control rats.

**CONCLUSIONS:** Our results indicate that wGE grafts are an effective method to restore (at least partially) individual skilled limb movements in the QA-lesioned rat and support the continuing development of cell transplantation as a potential future therapy for HD.

## **DONOR AGE DEPENDENT GRAFT DEVELOPMENT AND RECOVERY IN A RAT MODEL OF HD; CELLULAR AND BEHAVIOURAL ANALYSIS**

**Stefanie Schackel**, Anita Papazoglou, Guido Nikkhah, Máté Döbrössy

Department of Stereotactic and Functional Neurosurgery, Laboratory of Molecular Neurosurgery, University Hospital Freiburg, Germany

**BACKGROUND:** Clinical trials of fetal striatal grafts in HD patients have so far shown a range of outcomes from no effect to disease modifying capability. Amongst the many variables involved is the age of the donor embryo at the time of grafting. In this study we aim to shed more light on how embryonic striatal cells from different aged donors develop *in vivo*, what kind of cell populations they contain, how and for how long grafted cells proliferate, how they reconnect with the host, and how they influence behavioural recovery.

**METHODS:** 50 rats were used for the study of which 40 were lesioned unilaterally with QA (0.12M). 14 days later, animals received single cell suspension grafts equivalent of one whole ganglionic eminence (WGE) from E13 (n=10), E14 (n=10), E15 (n=10) donors, or remained lesioned only. Half of the animals in each grafted group were injected 3 times with BrdU (50mg/kg) following the next 48 hours to visualize cellular proliferation, and the other half received BrdU injections according to the same schedule 21 days post-grafting. All animals were tested on the Cylinder test, the Corridor test, and on drug-induced rotation at baseline, post-lesion/ pre-grafting, and at 6 and 10 weeks post-grafting. A week prior to perfusion, a sub-group in each grafted group received fluorogold injections into the ipsilateral globus pallidus to study graft re-innervation. Animals were perfused 12 weeks post-grafting and submitted to staining and image analysis.

**RESULTS:** The extent of functional recovery was shown to be dependent on i.) the age of the embryonic donor tissue; ii.) the context of the behavioural test; and iii.) the delay between the grafting and the testing. E13 tissue grafts gave the best overall outcome. Histological and morphometric data is currently being collected.

**CONCLUSIONS:** The functional data suggests that tissue from different donor age have different potential to promote functional recovery. In-depth histological analysis is currently underway to look at relation between functional outcome and graft characteristics and these results will be presented at NECTAR



**A PRELIMINARY REPORT ON OUTCOMES IN THE FRENCH  
LANGUAGE MULTI-CENTRE TRIALS IN HUNTINGTON'S DISEASE**

**Anne-Catherine Bachoud-Lévi**

## **GENETIC MODIFIERS FOR HUNTINGTON'S DISEASE**

**Lesley Jones**

Dept of Psychological Medicine, School of Medicine, Cardiff University, Heath Park.

The symptoms of Huntington's disease (HD) are variable and occur at different ages in different people. The CAG repeat in the HTT gene is a genetic modifier of the onset of HD. Not all of the variation in age of onset, however, can be attributed to the length of the CAG repeat: other factors are involved. This tells us two important things. Firstly, knowing someone's CAG repeat length does not allow accurate prediction of the age that someone will get HD. Secondly, other factors must influence the age at onset of HD. We know that having a particular set of gene variants can predispose people to common diseases such as heart disease or diabetes and there is evidence that multiple genes may influence the age at which people get HD. Gene variants can act to slow down the onset of disease (a protective effect) or speed it up (a promoting effect). Any gene which contains variation that slows down or speeds up onset of HD must lie in a biological pathway that affects the disease process. Such biological pathways are targets for therapies that aim to boost protective pathways and inhibit toxic ones. Looking for genetic modifiers in people is vital as it tells us directly about human HD.

## **PALLIDAL DEEP BRAIN STIMULATION (DBS) IN CHOREA HUNTINGTON**

**Jan Vesper, Wojtecki L, Wille C, Schnitzler A**

**Introduction:** At present, there is no effective treatment or cure for Huntington's disease (HD) patients. Therefore, neural stem cell transplantation seemed to offer a potential treatment for HD patients that may slow down this devastating illness. However there remain major concerns in transplantation. Therefore our group looked for alternatives, utilizing Deep Brain stimulation (DBS), based on the long-lasting successful treatment of other neurodegenerative movement disorders like Parkinson's disease (PD). Questions remained concerning the optimal target.

**Methods:** This phase I clinical trial is based on the hypothesis that deep brain stimulation of the internal pallidum can reduce choreatic symptoms in 3 HD patients. In addition, this trial should demonstrate which target point within the pallidum can be used effectively for specific features of HD in order to further refine this promising strategy for a phase II multicenter trial approach. Three consecutive cases with DBS of the Pallidum (Gpi/Gpe region) are reported. Electrodes were stereotactically implanted under general anesthesia, followed by the implantation of a neurostimulation system.

**Results:** No complications occurred. The coordinates for the active contacts in the Gpi/Gpe range were adapted to individual anatomical changes. Under DBS of the pallidum choreatic movements could be reduced by 50 to 80% (UHDRS). The quality of life, which was measured by ADL, was significantly improved in the three patients. Since the effects are delayed, the adjustment and testing of the remaining contacts took place in the course of 6 months postoperatively. The most effective active contacts were in projection of the border of Gpi and Gpe.

**Conclusion:** Systematic positive influence of DBS in Huntington's disease patients is reported for the first time. In the context of the following study it will have to be clarified whether the internal or the external part or other targets are suitable for DBS and which long-term results can be obtained. Implications for a potential MCT will be discussed.

**ENVIRONMENTAL AND TRAINING ASSOCIATED FACTORS IN  
TRANSPLANT-MEDIATED BRAIN REPAIR:  
DOES NEUROREHABILITATION HAVE A ROLE IN CELL  
REPLACEMENT THERAPY?**

**Máté Dániel Döbrössy**

Department of Stereotactic and Functional Neurosurgery, Laboratory of Molecular Neurosurgery, University Hospital Freiburg, Germany

Enriched environment, behavioural experience and grafting can each separately influence neuronal plasticity and recovery of function in animal models of brain damage. However, the mechanisms by which these factors interact, so that the environment or training might modify the survival, integration or function of grafted tissues is at present unknown. To improve the outcome following brain damage, cell replacement therapy must both make use of the endogenous potential for recovery of the host and optimize the external circumstances associated with any intervention. In particular, the observation that grafts can serve as the substrate for functional recovery following specific training protocols indicates that graft function and experience are not simply independent and additive, but fundamentally interactive.

Recent evidence will be presented showing that embryonic striatal grafts form functional connections with the host striatal circuitry capable of restoring stable synaptic transmission in an excitotoxic lesion model of Huntington's disease. The electrophysiology based study demonstrated that striatal grafts enable the expression of host-graft bi-directional synaptic plasticity, similar to the normal corticostriatal circuit. These results indicate that striatal grafts express synaptic correlates of learning, and thereby provide direct evidence of functional neuronal circuit repair, an essential component of 'functional integration'.

The growing data emerging from pre-clinical studies of plasticity demonstrated by the grafts in response to environmental and training stimuli has hitherto been almost totally neglected throughout the clinical application of cell therapy. The data discussed in the talk will suggest that a different approach might be required to maximize recovery: postoperative experiences, including rehabilitation with explicit behavioural retraining, could have marked direct, as well as positive secondary effects, on the integration and function of grafted cells in the host neural system.

## THE PHARMACOLOGICAL MODULATION OF GRAFT INDUCED DYSKINESIA IN THE TRANSPLANTED HEMI-PARKINSONIAN RAT

Gaynor Smith<sup>1</sup>, Emma Lane<sup>2</sup> and Steve Dunnett<sup>1</sup>

<sup>1</sup>Brain Repair Group, Cardiff University

<sup>2</sup>Welsh School of Pharmacy, Cardiff University

**Background:** The neural transplantation of embryonic dopamine rich cells into Parkinson's disease patients has led to symptomatic improvements. Clinical trials have shown that a proportion of patients develop debilitating dyskinesias in response to the graft, irrespective of L-dopa treatment, these hyperkinesias can be mimicked experimentally in rodents. The mechanistic similarity between graft induced dyskinesia (GID) and L-dopa induced dyskinesia (LID) is currently unknown. This may be elucidated by the pharmacological modulation of GID with substances known to affect LID in rodent models.

**Methods:** 32 rats were unilaterally lesioned with 6-OHDA, targeted to the median forebrain bundle. Rats received transplants of E14 ventral mesencephalon tissue, into the denervated striatum. Following neuronal maturation, animals were challenged with 2.5mg/kg of methamphetamine to elicit dyskinesia and rotational bias was recorded simultaneously. 16 selected rats were chosen with a range of abnormal inhibitory movements (AIMs) then given 2.5mg/kg of methamphetamine co-administered with a pharmacological challenge: - yohimbine (10mg/kg), naloxone (4mg/kg and 8mg/kg), amantadine (20mg/kg and 40mg/kg), SCH-22390 (0.05mg/kg and 0.2mg/kg), raclopride (0.5mg/kg and 2mg/kg), nafadotride (0.6mg/kg and 1mg/kg), WIN55,212-5 (1mg/kg and 2.5mg/kg), MK-801 (0.03mg/kg and 0.3mg/kg). Non-dyskinetic animals were used as controls.

**Results:** AIMs decreased with SCH-22390 and raclopride, and to a lesser extent amantidine and nafadotride in the dyskinetic group, and were unchanged in non-dyskinetic animals. AIMs in response to MK-801 were increased and a reversion in rotation was observed. GID was unchanged by yohimbine, naloxone and WIN55,212-5.

**Conclusions:** The dyskinesia caused by L-dopa in lesioned models and methamphetamine in grafted models is differently modulated by pharmacological agents, initiative of an alternate mechanism of AIM development. The glutamate and dopaminergic systems are likely to have a fundamental role in the development of GID and are not influenced by opioid, adrenergic or cannabinoid systems.

## **PREDICTIVE VALUE OF SEVERITY OF PREOPERATIVE L-DOPA-INDUCED DYSKINESIA FOR THE DEVELOPMENT OF GRAFT-INDUCED DYSKINESIA IN THE RAT PARKINSON MODEL**

**Joanna García**<sup>1</sup>, Thomas Carlsson<sup>3</sup>, Guido Nikkhah<sup>1</sup>, Christian Winkler<sup>2</sup>

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<sup>3</sup>Dept. of Experimental Neurology, Phillips University Marburg, Marburg, Germany

**BACKGROUND/AIM:** In Parkinson's disease, intracerebral transplantation of fetal dopamine cells has been analyzed as a new treatment option. While some patients showed improvements of motor function and reductions of motor fluctuations such as L-DOPA-induced dyskinesia (LID), some patients developed a new type of off-medication dyskinesia now known as graft-induced dyskinesia (GID). The reasons for the development of GID are unclear, but since some of the patients with GID had high preoperative LID-scores, we decided to analyze the predictive value of preoperative LID for the development of GID in the rat Parkinson model.

**METHODS:** All animals first received unilateral 6-OHDA lesions into the MFB in order to induce complete dopamine-denervating lesions. Amphetamine-induced rotation (2,5mg/kg i.p.) and cylinder test were performed to evaluate the severity of the lesion. Daily L-DOPA injections (6mg/kg s.c. + benserazide, 10mg/kg) were performed for 4 weeks to induce stable dyskinesia. LID was maintained thereafter by injection of L-DOPA twice weekly throughout the experiment using the same dose. Animals received transplants of fetal ventral mesencephalon into the striatum. Transplant-induced changes in amphetamine-induced rotation were analyzed at 18 weeks post grafting, and changes in cylinder test were assessed at 3 and 15 weeks post grafting. LID was analyzed 2, 4, 12, 16 and 24 weeks post grafting. GID induced by amphetamine (1,5mg/kg i.p.), was analyzed at 5 and 17 weeks after transplantation. Animals were perfused at 24 week post transplantation. Immunohistochemistry was performed for TH-positive grafted cells and fibers. Number of grafted cells was assessed using stereology. Striatal optical fiber density was assessed in the whole or in the caudolateral striatum.

**RESULTS:** All grafted groups showed significant amelioration of amphetamine-induced rotation and significant improvements in the cylinder test. LID was significantly reduced in animals with severe preoperative LID, and remained low in grafted animals with low preoperative LID. GID was pronounced in animals with severe preoperative LID and less severe in animals with low preoperative LID values.

**CONCLUSIONS:** Our data indicate, that the severity of preoperative LID predicts the risk for the development of GID in the rat Parkinson model. This suggests that patients with severe LID should not receive intracerebral dopamine grafts.

## SEROTONIN NEURON-DEPENDENT AND –INDEPENDENT REDUCTION OF DYSKINESIA BY 5-HT<sub>1A</sub> AND 5-HT<sub>1B</sub> RECEPTOR AGONISTS IN THE RAT PARKINSON MODEL

**Manolo Carta**, Ana Muñoz, Thomas Carlsson, Elisabetta Tronci, Deniz Kirik, Anders Björklund

**Background:** 5-HT<sub>1</sub> receptor agonists have been shown to reduce dyskinesia in the rat and monkey models of L-DOPA-induced dyskinesia. However, multiple mechanisms may account for this effect. Activation of 5-HT<sub>1</sub> receptors at pre-synaptic level is suggested to result in reduction of dopamine release from the serotonin terminals, and thus prevent abnormal activation of striatal dopamine receptors. Activation of post-synaptic 5-HT<sub>1</sub> receptors, by contrast, has been shown to result in decreased glutamate and GABA release, which may also contribute to the antidyskinetic effect.

**Aim:** To unveil the relative contribution of these mechanisms, we have investigated the effect of increasing doses of 5-HT<sub>1A</sub> and 5-HT<sub>1B</sub> receptor agonists on dyskinesia induced by either L-DOPA or apomorphine. In contrast to L-DOPA-induced dyskinesia, which depends on the release of L-DOPA-derived DA from serotonin terminals, the dyskinesias induced by apomorphine are elicited by a direct action on post-synaptic DA receptors, thus by-passing the serotonin neurons.

**Results:** The results showed that L-DOPA-induced dyskinesia was dampened already at low doses of 5-HT<sub>1</sub> agonists, while serotonin neuron-independent reduction of dyskinesia required higher doses. Removal of the serotonin innervation suppressed L-DOPA induced dyskinesia, but neither affected apomorphine-induced dyskinesia nor the inhibiting effect of 5-HT<sub>1</sub> agonists on dyskinesia induced by the dopamine direct agonist, suggesting that such effect is independent on activation of pre-synaptic 5-HT<sub>1</sub> receptors.

**Conclusions:** The results are important for the understanding of the mechanisms accounting for the antidyskinetic effect of 5-HT<sub>1</sub> agonists, and support the relevance of future clinical investigations to test the feasibility of this approach to treat dyskinesia in Parkinson's disease patients.

## HIGH SEROTONIN TRANSPORTER BINDING IN TWO PATIENTS WITH PARKINSON'S DISEASE AND STRIATAL FOETAL MESENCEPHALIC GRAFTS. IMPLICATIONS FOR GRAFT INDUCED DYSKINESIA?

Marios Politis, Kit Wu, Clare Loane, Niall P.Quinn, David J.Brooks, Stig Rehncrona, Olle Lindvall, Anders Bjorklund and Paola Piccini

**Background:** Presynaptic dopamine (DA) terminal restoration and dopamine release has been monitored in the past in the grafted striatum of Parkinson's disease (PD) patients. 18F-dopa Ki levels have been reported slightly below normal levels 10 years after transplantation in grafted putamen with very small changes after the 4<sup>th</sup> year (Piccini et al., 1999).

It was recently shown that in parkinsonian rats, serotonin grafts induced a worsening in the severity of L-DOPA induced dyskinesias (Carlsson et al., 2007) as DA can be transported into 5-HT neurons via 5-HTT (Saldana and Barker, 2004) and lead to dysregulated release of DA in the synapse, contributing to dyskinesias.

**Aims:** To assess in vivo in two PD patients transplanted in Lund (Serial No: 7 and 15) the 5-HTT availability and compare it with a group of patients with advanced PD and L-DOPA induced dyskinesias (n=6; 5M:1F; Disease duration =  $10.92 \pm 4.84$ ; mean  $\pm$  SD) and a group of normal controls (n=8, 6M: 2F) .

**Methods/Techniques:** We have studied two PD patients that received foetal mesencephalic tissue: Grafted PD 1 (Serial No: 7): Received transplant 15 and 16.5 years ago in the R and L Putamen, respectively. UPDRS<sub>TOTAL</sub> =37, UPDRS<sub>PART III</sub> =13, AIMS =19 and PDQ-39 = 36.

Grafted PD 2 (Serial No: 15): Received transplant 13 years ago bilaterally in Caudate and Putamen. UPDRS<sub>TOTAL</sub>=17, UPDRS<sub>PART III</sub> =7, AIMS =11 and PDQ-39 = 28

Both subjects were not on L-DOPA.

We have used PET with 11C-DASB, a selective radioligand for the 5-HTT and marker for the serotonin terminals and we have calculated Caudate and putaminal BP<sub>ND</sub> using an input function derived from the receptor-free posterior cerebellar cortex. Volume of Distribution Ratio and BP<sub>ND</sub> were calculated as described by Logan et al (1996) and Ginovart et al (2001), respectively.

### Results/Outcome:

11C – DASB	Normal Controls (n=8)	PD Controls (n=6)	Grafted PD 1 (No 7) (L+R put)	Grafted PD 2 (No 15) (L+R caud/L+R put)
Caudate BP <sub>ND</sub> (mean $\pm$ SD)	1.41 $\pm$ 0.17 (Range: 1.11 – 1.68)	0.99 $\pm$ 0.23 (Range: 0.79 – 1.30)	1.29	3.03
Putamen BP <sub>ND</sub> (mean $\pm$ SD)	1.39 $\pm$ 0.14 (Range: 1.15 – 1.63)	1.04 $\pm$ 0.26 (Range: 0.62 – 1.29)	3.70	2.24

**Conclusions:** We report high levels of 5-HTT binding in striatal sites of two PD patients that had received foetal mesencephalic tissue grafts and had developed graft-related “off” dyskinesias in comparison to PD patients with L-DOPA induced dyskinesias. Interestingly, dyskinesia score was higher in the transplanted PD subject with higher 5-HTT binding in the Putamen. It is possible that the mishandling of dopamine from 5-HT neurons present in the grafted tissue may contribute to development of graft-related dyskinesia.

**FRIDAY 27<sup>TH</sup> NOVEMBER PM**

**SESSION 5**

**PARKINSON'S DISEASE**

**Session 5**

**Parkinson's disease**

14.00-14.30 Datablitz 4

14.30-14.55 Angela Cenci-Nilsson Our current understanding of the mechanisms of L-DOPA-induced dyskinesias in Parkinson's disease

14.55-15.20 Emma Lane Progress in understanding graft-induced dyskinesia

15.20-15.45 Paola Piccini The development of imaging strategies for refining clinical neural transplantation trial

15.45-16.15 Datablitz 5

**16.15-16.35 Coffee Break**

16.35-17.35 Roger Barker PD and clinical trials – where do we go from here?  
Discussion

17.35-17.50 NECTAR business meeting

**19.30 Conference dinner**

**Datablitz 4:**

- 1 INVESTIGATING THE POTENTIAL TO GROW AXONAL PROJECTIONS IN AN IMPLANTATION CAPILLARY *EX VIVO*, AND TRANSPLANTING IN TECT NEURONAL "WIRES"  
**Yiwen Liu**
- 2 TWO-STEP GRAFTING – A NEW METHOD TO ENHANCE CELL SURVIVAL AND STUDY GRAFT DEVELOPMENT IN A 6-OHDA RAT MODEL OF PARKINSON'S DISEASE (PD).  
**Fabian Buechele**
- 3 NIGROSTRIATAL EXPRESSION OF FETAL ANTIGEN-1/DELTA-LIKE HOMOLOGUE IN ADULT RAT AND IN A MODEL OF PARKINSON'S DISEASE  
**Angélique Ducray**
- 4 FURTHER DEVELOPMENT OF CELL-BASED TRANSPLANTATION IN A RAT MODEL OF PARKINSON'S DISEASE  
**Andre Nobre**
- 5 THE ROLE OF ENDOGENOUS FGF-2 DURING DEVELOPMENT OF THE SUBSTANTIA NIGRA OF THE MOUSE  
**Andreas Ratzka**
- 6 WNT5A REGULATES MIDBRAIN DOPAMINERGIC AXON MORPHOGENESIS  
**Clare L Parish**

**Datablitz 5:**

- 1 POST-GRAFT RECOVERY ON THE STAIRCASE-TEST IN THE RODENT PARKINSON'S DISEASE MODEL: DOES PELLET DENSITY HAVE AN IMPACT?  
**Karina Kohn Cordeiro**
- 2 THE A9 DOPAMINERGIC NEURON COMPONENT IN GRAFTS OF FOETAL VENTRAL MESENCEPHALON IS AN IMPORTANT DETERMINANT OF GRAFT-INDUCED FUNCTIONAL RECOVERY IN A RAT MODEL OF PARKINSON'S DISEASE  
**Shane Grealish**
- 3 POTENTIAL OF RAT BONE MARROW-DERIVED MESENCHYMAL STEM CELLS AS VEHICLES FOR DELIVERY OF NEUROTROPHIC SUPPORT TO THE PARKINSONIAN RAT BRAIN  
**Teresa Moloney**
- 4 IN-VITRO CHARACTERIZATION OF VENTRAL MESENCEPHALIC TISSUE DERIVED FROM MEDICAL TERMINATION OF PREGNANCY  
**Ulrike Weyrauch**
- 5 CHARACTERIZATION OF LEWY BODY PATHOLOGY IN 12- AND 16 YEAR OLD INTRASTRIATAL MESENCEPHALIC GRAFTS SURVIVING IN A PATIENT WITH PARKINSON'S DISEASE  
**Jia-Yi Li**
- 6 HUMAN-DERIVED VENTRAL MESENCEPHALIC DOPAMINERGIC GRAFTS IN THE 6-OHDA RAT MODEL OF PARKINSON'S DISEASE  
**Anika Rath**

## INVESTIGATING THE POTENTIAL TO GROW AXONAL PROJECTIONS IN AN IMPLANTATION CAPILLARY *EX VIVO*, AND TRANSPLANTING IN TACT NEURONAL “WIRES”

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**BACKGROUND:** Neural circuits in the adult mammalian central nervous system (CNS) are difficult to repair via cell transplantation. Axon elongation and pathfinding is made difficult by the expression of inhibitory molecules, or the paucity of growth-promoting cues, in the host CNS.

**AIM:** To by-pass the negative effects of the host CNS, we have recently begun exploring the possibility to grow and / or implant donor neurons after they have established lengthy axonal projections.

**METHODS:** First, to determine the feasibility of establishing neuronal projections in an implantation capillary, dissociated E13 rat VM cells, or pieces of VM tissue, were mixed with rat tail collagen I, aspirated into a glass capillary, and grown for 7 days *in vitro*. Second, to determine if tissue can be implanted without compromise to its structural integrity; embryonic tissue was loaded into a glass capillary and displaced (while being viewed via video microscopy) into a transparent gelatin matrix using a implantation device designed to “lay out” tissue during delivery.

**RESULTS:** Dissociated VM cells, mixed with collagen, show a dispersed distribution and undirected growth pattern throughout the implantation capillary, while dissected VM tissue (placed at the end of an implantation capillary) extended axons through the collagen in a directed manner. For delivery, tissue can be readily loaded into an implantation capillary and “laid out”, in tact, without compromise to the tissue’s cytoarchitecture and orientation.

**CONCLUSIONS:** In the future, small segments of neural circuits or tissue could be grown *in vitro* or harvested from primary sources, and potentially implanted (intact) into a recipient brain; allowing for replacement of short circuits in the adult rodent brain.

## TWO-STEP GRAFTING – A NEW METHOD TO ENHANCE CELL SURVIVAL AND STUDY GRAFT DEVELOPMENT IN A 6-OHDA RAT MODEL OF PARKINSON'S DISEASE (PD)

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**Background/Aims:** Transplantation studies in the 6-OHDA rat model of PD have shown that only 8-10% of the grafted embryonic ventral mesencephalic (VM) dopaminergic (DA) cells survive. In order to increase this rate, we establish a new two-step grafting protocol where a standard amount of cells is divided in half and grafted in 2 separate sessions with a defined time interval. We furthermore give an insight into the underlying mechanisms by altering the time intervals, the amount of cells in the 1<sup>st</sup> graft and by evaluating the 2 grafts independently using GFP<sup>+</sup> cells.

**Methods:** 48 6-OHDA-lesioned rats were divided into 3 two-step grafting groups, each with a time interim of 2, 5 or 9 days between the 2 transplantation sessions. Each group was sub-divided in 2 sub-groups receiving either  $2 \times 10^5$  (low cell number groups: 2dL, 5dL, 9dL) or  $4 \times 10^5$  (high cell number groups: 2dH, 5dH, 9dH) GFP<sup>-</sup> VM cells in the 1<sup>st</sup> grafting session. For the 2<sup>nd</sup> transplantation, all groups received the equal amount of  $2 \times 10^5$  GFP<sup>+</sup> cells.

As control, 2 standard transplantation groups (standard low/high cell number groups, StL/StH) were grafted with the same constellation of cells in a single operation session.

Transplantation effects were evaluated by drug-induced rotation tests 2 and 6 weeks after the 1<sup>st</sup> grafting. The animals were sacrificed 8 weeks after transplantation and graft survival was evaluated by stereology.

**Results:** In the rotation test all transplanted groups showed significant compensation from the lesion. Morphological analysis showed a better survival of DA cells in the 2d groups in comparison to all other transplantation groups. The cell number in the 2dH group was significantly higher than in the 5dH (+ 50%), the 9dH (+ 68%) and the StH standard group (+ 47%).

In addition, as an incidental finding, some grafts showed intense growth of GFP<sup>+</sup> (2<sup>nd</sup> graft derived) vessels present in 87.5% of the 2dH, 60% of the 2dL and 42,9% of the 5dH transplants. These vessel networks only formed in regions where both grafts overlap and were completely absent in the standard and the 9d groups.

**Conclusion:** Our findings show that two-step grafting with a 2 days time interval significantly increases DA cell survival in comparison to the standard protocol. As a potential mechanism we found an increased donor-derived vessel formation which only occurs under specific conditions.

## NIGROSTRIATAL EXPRESSION OF FETAL ANTIGEN-1/DELTA-LIKE HOMOLOGUE IN ADULT RAT AND IN A MODEL OF PARKINSON'S DISEASE

**Angélique Ducray**, RÉMY LIECHTI, PIA JENSEN#, STEFANO DI SANTO, CHARLOTTE HARKEN JENSEN##, ANDREAS RAABE, MORTEN MEYER# AND HANS RUDOLF WIDMER.

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**Background:** Fetal antigen 1/delta-like 1 homologue (Dlk1), encoded by the gene DLK1, is a transmembrane protein belonging to the epidermal growth factor superfamily. Dlk1 is expressed in the ventral mesencephalon, and it has been suggested as a potential alternative marker protein in dopaminergic neurons, which is of particular interest in relation to Parkinson's disease. At present, however, functions of cerebral Dlk1 remain largely unknown. Hence, the present study aimed at characterizing the expression pattern of Dlk1- immunoreactive (-ir) cells in the adult rat nigrostriatal system as well as in the 6-hydroxydopamine (6-OHDA) rat model of Parkinson's disease.

**Results:** Immunohistochemical analyses revealed first that Dlk1 was expressed by several cells in the midbrain. Co-localization experiments showed that most of the nigral tyrosine hydroxylase-ir cells also expressed Dlk1. Furthermore, Dlk1-ir cells were detected in ventricular and subventricular zone and scattered throughout the striatum. Unilateral striatal or middle forebrain bundle (MFB) injections of 6-OHDA resulted in a significant loss of dopaminergic neurons as well as Dlk1-ir cells in the substantia nigra pars compacta. Accordingly, we observed a disappearance of dopaminergic and Dlk1-ir fiber innervation in the ipsilateral striatum, suggesting that Dlk1 is expressed in projection neurons. Interestingly, quantitative analyses revealed that striatal densities of Dlk1-ir cells were significantly higher on the side of the 6-OHDA lesion compared to unlesioned side (76% and 68% for striatal and MFB lesions, respectively).

**Conclusion:** In conclusion, our findings identify Dlk1 as a competent supplementary marker for dopaminergic neurons in the adult substantia nigra, and demonstrate that Dlk1 expression is dynamically regulated in response to lesions of the nigrostriatal system.

## FURTHER DEVELOPMENT OF CELL-BASED TRANSPLANTATION IN A RAT MODEL OF PARKINSON'S DISEASE

Andre Nobre <sup>a,b</sup>, Ieva Kalve <sup>a,b</sup>, Andreas Ratzka <sup>a</sup>, Maike Wesemann <sup>a</sup>, Claudia Grothe <sup>a,b</sup>

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**BACKGROUND:** Cell replacement strategies represent a potential alternative treatment to restore lost functions in Parkinson's disease. A large number of experimental studies have shown efficacy of implanted embryonic mesencephalic tissue into the dopamine-denervated striatum. Limitations of the method include limited supply of donor cells and the low survival of grafted cells.

**AIMS:** To increase the number of dopaminergic (DA) neurons surviving the transplantation procedure, we optimized cell culture conditions and transfection procedure to over-express neurotrophic factors non-virally.

**METHODS:** Neuronal progenitor cells isolated from the ventral mesencephalon of E12 rat embryos were differentiated into dopaminergic (DA) neurons *in vitro*, nucleofected with expression plasmids and transplanted into unilateral 6-OHDA rat model of Parkinson's disease (Timmer et al. Neurobiol. Dis. 2006; Cesnulevicius et al. Stem Cells 2006).

**RESULTS:** Nucleofection of an enhanced green fluorescence protein (EGFP) plasmid resulted in robust and long-term expression for at least 3 weeks *in vitro* and 12 weeks after transplantation *in vivo*. Moreover modification of our previous cell culture protocol resulted in higher survival and advanced maturation of the transfected neurons. In addition, stereological quantification of grafted cells revealed an improved number of DA neurons with the modified protocol.

**CONCLUSIONS:** This newly established protocol will allow studying the effects of candidate genes on differentiation and survival of transplanted DA cells. In this regard plasmids expressing neurotrophic factors are currently under investigation.

## THE ROLE OF ENDOGENOUS FGF-2 DURING DEVELOPMENT OF THE SUBSTANTIA NIGRA OF THE MOUSE

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**BACKGROUND:** Loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc) leads to characteristic symptoms of Parkinson's disease. Understanding the regulation of SNpc development may contribute to an improvement of therapeutic approaches (e.g. cell replacement strategies). Previously we have analyzed fibroblast growth factor 2 (FGF-2) mutant mice at the adult stage and found an increased number of tyrosine hydroxylase immunoreactive (THir) neurons in FGF-2 deficient mice and a reduced number of THir neurons in FGF-2 over-expressing animals (Timmer et al., *J. Neurosci.* 27, 2007).

**AIMS:** To elucidate the role of endogenous FGF-2 on DA neuron development we are analyzing the SNpc of embryonic and neonatal stages of FGF-2 deficient mice to investigate: 1.) at which developmental stage differences in the SNpc become evident and 2.) if the loss of FGF-2 changes the expression of other members of the FGF gene family.

**METHODS:** THir neurons in the SNpc of FGF-2 deficient and wild-type animals were quantified using design-based (3D) stereological method (CASTgrid software). Expression levels of FGF-family members were characterized by quantitative RT-PCR.

**RESULTS:** The stereological quantification of newborn FGF-2 deficient animals revealed a 20% higher THir neuron number in SNpc. Quantitative RT-PCR assays for all 22 FGF family members were established. First results of the adult ventral mesencephalon and striatum revealed no changes (beside of FGF-2) in expression levels of other FGFs in FGF-2 deficient animals.

**CONCLUSIONS:** Our data suggests that FGF-2 might influence embryonic DA development (proliferation, differentiation or migration) rather than postnatal apoptotic events. Therefore we have started to analyse E14.5 animals on morphological and molecular level.

## WNT5A REGULATES MIDBRAIN DOPAMINERGIC AXON MORPHOGENESIS

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**Background:** Current cell replacement therapy (CRT) for Parkinson's disease relies on ectopic (intra-striatal) grafts because homotypic (intranigral) grafts survive poorly and form inadequate connections. Ectopic grafts failure to adequately reinnervate the striatum and restore normal brain circuitry, thereby only partially restoring motor function. Successful CRT will only be achieved by promoting homotypic graft survival, axonal growth and integration into the host circuitry. We contend that this can be accomplished by understanding the guidance cues involved during development.

**Aim:** To examine the role of the morphogen Wnt5a in regulating dopamine (DA) axon morphogenesis. **Methods:** We mapped the temporal-spatial expression of Wnt5a relative to the developing midbrain DA pathways and examined the responsiveness of DA neurites to Wnt5a. We used Wnt5a<sup>-/-</sup> mice to validate a role of Wnt in DA axon morphogenesis.

**Outcomes:** We illustrate a temporal-spatial expression of Wnt5a that overlaps with the development of DA axon. In ventral midbrain (VM) primary cultures, enriched in DA neurons, we show that Wnt5a promotes neurite growth and alters DA neuron complexity, at a time when axons are exiting the VM. Later in development, when DA axons are approaching their striatal target, Wnt5a causes neurite retraction. Antagonism experiments reveal that these effects are mediated via the atypical receptor tyrosine kinase, Ryk resulting in downstream activation of the non-canonical planar cell polarity Wnt pathway. Further, we show that Wnt5a repels DA neurites in explant cultures. The importance of Wnt5a in DA axon morphogenesis was verified in Wnt5a<sup>(-/-)</sup> mice, where fasciculation of the medial forebrain bundles was disrupted as well as the density of DA neurites and striatal terminals.

**Conclusion:** These results identify a novel role Wnt5a in DA axon morphogenesis, findings that may be important in restoring DA circuitry following homotypic transplants in PD.

## **OUR CURRENT UNDERSTANDING OF THE MECHANISMS OF L-DOPA-INDUCED DYSKINESIAS IN PARKINSON'S DISEASE**

**M. Angela Cenci.**

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L-DOPA remains the most effective treatment for Parkinson's disease (PD), but causes dyskinesia (abnormal involuntary movements) in the vast majority of the patients. During the past few years, an increasing number of laboratories have pursued the basic mechanisms of L-DOPA-induced dyskinesia using animal models of this movement disorder. A growing body of studies have uncovered many neurochemical, molecular, and cellular alterations in the basal ganglia in dyskinetic animals. This lecture will attempt to integrate these studies into a unifying pathophysiological picture, where different cellular mechanisms converge to generate either presynaptic abnormalities in dopamine release and clearance, or abnormal postsynaptic responses in dopaminergic cells (striatal neurons in particular). The expression of dyskinesia is attributed to altered glutamatergic, GABAergic and peptidergic transmission in the output nuclei of the basal ganglia, impacting on the activity of thalamo-cortical networks and brainstem premotor structures.

## **PROGRESS IN UNDERSTANDING GRAFT-INDUCED DYSKINESIA**

**Emma Lane**

Welsh School of Pharmacy, Cardiff University

Neural transplantation has shown significant promise as a therapy for Parkinson's disease, some patients showing significant clinical benefit. However, the identification of abnormal involuntary movements in some transplanted patients, now termed graft-induced dyskinesia, has been a major setback in the development of this approach. Determining the factors that influence the expression of GID, and its underlying cause has been a major focus for proponents of transplantation. Different factors have been considered as the cause of these movements at all levels of the process, from tissue dissection and manipulation to patient selection. Significant progress has been made through further evaluation of the available clinical data and the development of an animal model of GID. These data are stimulating confidence in the field that new clinical trials can be designed to maximize therapeutic benefit and avoid motor side effects following transplantation.

## THE DEVELOPMENT OF IMAGING STRATEGIES FOR REFINING CLINICAL NEURAL TRANSPLANTATION TRIAL

**Paola Piccini, MD PhD FRCP**

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Functional imaging with  $^{18}\text{F}$ -dopa and positron emission tomography (PET) have been used since the late 80' to objectively monitor survival of human fetal dopamine (DA) neurons grafted in the striatum of Parkinson's disease (PD) patients. In more recent double-blind sham-surgery controlled clinical trials  $^{18}\text{F}$ -dopa uptake was shown to be unchanged or reduced in patients who had sham surgery and significantly increased in comparison to baseline in patients who had received grafted cells. However, in several cases there was a lack of correlation between increases in  $^{18}\text{F}$ -dopa uptake and improvement in clinical symptoms. This could be explained by the fact that while  $^{18}\text{F}$ -dopa uptake robustly reflects DA storage capacity in the dopamine terminals/cells it may not be an adequate indicator of dopamine terminals/cells function such as their ability to release DA in the synaptic cleft. It has become therefore clear that other imaging techniques need to be employed to monitor graft function.

$^{18}\text{F}$ -dopa studies are still important as part of the assessment in transplantation trials as this technique can provide an useful tool for screening patients which are not suitable for transplantation i.e. patients whose dopamine deficit extends to the ventral striatum.

Displacement studies with double  $^{11}\text{C}$ -Raclopride scans and a pharmacological challenge have shown that it is possible to assess the capacity of releasing endogenous dopamine from grafted cells, but these studies are difficult to perform in large clinical trials. Activation studies with  $\text{H}_2^{15}\text{O}$  PET or functional MRI (fMRI) can be used to assess the effect of the transplanted dopamine cells on the basal-ganglia-cortical networks and the relevance of the restoration of these circuitries for the clinical outcome.

Imaging techniques can also provide some insight into the mechanisms underlying off-dyskinesias which can arise following transplantation. One possible hypothesis indicates that the mishandling of DA by 5HT neurons in the graft could be responsible for the dyskinesias. PET with a specific tracer for the 5HT transporter (SERT) will allow to study the density of 5HT neurons within the graft.

Finally, functional imaging can be used to monitor the inflammatory processes such as increases in microglia activation surrounding the grafted cells and whether these processes interfere with a good clinical outcome.



## POST-GRAFT RECOVERY ON THE STAIRCASE-TEST IN THE RODENT PARKINSON'S DISEASE MODEL: DOES PELLET DENSITY HAVE AN IMPACT?

**Karina Kohn Cordeiro**<sup>1,2</sup>, Anita Papazoglou<sup>1</sup>, Wei Jiang<sup>1</sup>, Máté Döbrössy<sup>1</sup>, Guido Nikkhah<sup>1</sup>

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**BACKGROUND:** The Staircase test is a common behavior test established to measure side-specific deficits in skilled paw reaching in rodents. Currently there is a discrepancy in the literature on whether performance in this paradigm is or is not sensitive to recovery mediated by VM grafts.

**AIM:** To evaluate the influence of pellet density on the graft-mediated functional recovery in the rodent model of PD.

**METHODS:** 53 rats were pre-trained over 22 days on the Staircase test with a configuration of 8 pellets in each of the 6 wells bilaterally; later 43 rats received unilateral 6-OHDA lesions of the MFB. A month later, half of the lesioned animals received E14 VM grafts into the dopamine depleted striatum. At this stage, the animals were subjected to re-testing over 25 days on the Staircase test under one of two configurations: bilaterally either with 10 pellets in each of the 6 wells (HIGH; 10x6 BL) or bilaterally with 2 pellets per each of the 6 wells (LOW; 2x6 BL). A subsequent testing session was done under the same pellet configuration but under forced choice conditions, whilst a final testing session crossed over the testing configurations. Animals were also tested on the Corridor and the Cylinder test, and subjected to drug-induced rotation.

**RESULTS:** Graft-mediated functional recovery was observed in the pellets taken criteria only under the HIGH pellet configuration (10x6) during the bilateral and the forced choice condition, as well as after the cross over. When tested under the LOW configuration, the graft provided no measurable benefit. The presence of VM grafts reduced lateralised motor deficits in the Cylinder test, the adjacent version of the Corridor test, and drug-induced rotation.

**CONCLUSION:** Our results confirm that VM transplants in the PD model are able to partially restore skilled forelimb motor deficits. However, this was only observed on the HIGH condition testing. The study suggests that pellet density has an impact on whether graft-mediated recovery is observed or not.

## THE A9 DOPAMINERGIC NEURON COMPONENT IN GRAFTS OF FOETAL VENTRAL MESENCEPHALON IS AN IMPORTANT DETERMINANT OF GRAFT-INDUCED FUNCTIONAL RECOVERY IN A RAT MODEL OF PARKINSON'S DISEASE

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**BACKGROUND:** A crucial aspect when pursuing alternative cell sources, such as embryonic stem cells, for cell replacement therapy in Parkinson's disease (PD) is the need to identify the optimal cells that can provide functional repair and integration in the denervated host brain. Grafts of foetal ventral mesencephalon (VM) contain a mixture of midbrain dopaminergic (DAergic) neuron subtypes; including those of the A10 (ventral tegmental area) and A9 (substantia nigra) cell groups.

**AIM:** In order to understand the importance of A9 neurons in functional recovery following transplantation, relative to those of the A10 group, we have conducted grafting experiments using foetal VM from the Pitx3-GFP knock-in mouse.

**METHODS:** Homozygous mice (Pitx3-GFP<sup>GFP/GFP</sup>) display a prominent loss of A9 neurons while the A10 neurons are relatively spared, whereas heterozygous mice (Pitx3-GFP<sup>WT/GFP</sup>) are similar to wild types. By transplanting VM dissected at E12.5 sourced from either Pitx3-GFP<sup>WT/GFP</sup> or Pitx3-GFP<sup>GFP/GFP</sup> embryos, into the striatum of 6-hydroxydopamine lesioned rats, we are able to generate grafts of a mixed A9/A10 composition or essentially A10 pure grafts, respectively. The influence the different graft compositions have on behaviours such as amphetamine-induced rotations and the cylinder test was assessed at 8 and 12 weeks post-transplantation, prior to termination for histological analysis.

**RESULTS:** The transplanted GFP reporter cells have allowed us to illustrate graft-derived DAergic innervation of the host striatum and we have observed that the Pitx3-GFP<sup>GFP/GFP</sup> grafts, lacking A9 neurons, innervate poorly, relative to the Pitx3-GFP<sup>WT/GFP</sup> grafts. This lack of innervation is also reflected in the rotation test, where Pitx3-GFP<sup>GFP/GFP</sup> grafts failed to correct the DAergic deficit, and these animals also displayed no recovery in the cylinder test.

**CONCLUSION:** These results highlight the impact that midbrain DAergic neuronal subtype has on graft-induced recovery following transplantation in a PD animal model.

## POTENTIAL OF RAT BONE MARROW-DERIVED MESENCHYMAL STEM CELLS AS VEHICLES FOR DELIVERY OF NEUROTROPHIC SUPPORT TO THE PARKINSONIAN RAT BRAIN

Teresa Moloney, Thomas Ritter, Frank Barry, Linda Howard and Eilís Dowd

**Background:** Bone marrow derived mesenchymal stem cells (MSCs) constitutively express neurotrophic factors including glial cell-line derived neurotrophic factor (GDNF) and may be capable of providing long term neurotrophic support to the Parkinsonian brain following direct transplantation to the central nervous system (CNS). Moreover, MSCs can be virally transduced *ex vivo* to over-express GDNF and may prove useful as vehicles to deliver neurotrophic support to the degenerating brain thereby circumventing debate surrounding delivery of virus directly to the CNS.

**Aims:** This study sought to determine the ability of normal MSCs and MSCs retrovirally transduced to over-express GDNF to protect against a neurotoxic insult *in vivo* at both the behavioural and neuro-anatomical level.

**Methods:** Rats received intrastriatal transplantation of either transplantation medium, normal MSCs or GDNF-transduced MSCs and were challenged with a unilateral intrastriatal 6-hydroxydopamine lesion four days later. Animals were subsequently tested for emergence of lateralised motor deficits and this was followed post-mortem by quantitative tyrosine hydroxylase immunohistochemistry.

**Results:** MSCs and GDNF-transduced MSCs did not ameliorate behavioural asymmetry and were unable to protect against degeneration of the dopaminergic cell bodies in the substantia nigra. However, dense tyrosine hydroxylase immunoreactivity was found surrounding the GDNF-transduced MSCs in the striatum indicating that these cells were capable of exerting a trophic effect on the surviving nigrostriatal neurons.

**Conclusions:** Although neither transplantation of MSCs nor GDNF-transduced MSCs conveyed functional or neuro-anatomical protection against a neurotoxic insult, the local trophic effect of the GDNF-transduced MSCs suggests that MSCs hold potential as vehicles to deliver neurotrophic support to the brain. Strategies aimed at improving the post-transplantational survival of MSCs may improve their neurotrophic potential and increase their candidacy as vehicles for gene delivery to the CNS.

## IN-VITRO CHARACTERIZATION OF VENTRAL MESENCEPHALIC TISSUE DERIVED FROM MEDICAL TERMINATION OF PREGNANCY

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**Background:** Medical terminations of pregnancy (MTOPs) are increasingly replacing surgical terminations of pregnancy (STOPs) as the method of choice in most hospitals in the UK.

The work presented here addresses the relative poverty of knowledge regarding the use of this tissue and its capacity to produce viable dopaminergic (DA) neurons for future human transplantation.

**Methods:** Ventral mesencephalic (VM) and cortical control tissues from routine MTOPs ( $n = 13$ ) were cultured and different parameters were examined including crown-to-rump length (CRL), age and viability at dissection; optimal culturing substrate; total cell and tyrosine hydroxylase immunoreactive (TH-ir) cell yields as well as effects of damage at collection on TH-ir cell survival.

**Results:** When specimens that showed signs of decay at dissection were excluded, excellent survival was achieved both of VM and cortical cultures on a standard Poly-L-Lysine substrate with best survival after 3 days. CRL at dissection was found to be a valuable predictor of TH-ir survival in vitro: embryos at donor ages when most DA neurons have undergone final differentiation (week 8 - 9 post-menstrual; 18 – 28 mm CRL) showed the highest yields of up to 200,000 TH-ir neurons per VM, with VMs from both smaller and larger embryos producing significantly fewer TH-ir neurons in vitro ( $F_{2,8}=18.27$ ;  $p<0.01$ , one-way ANOVA).

**Conclusion:** In conclusion, VM DA neurons can survive drug-induced termination and may be a promising source of cells for transplantation into Parkinson's disease patients.

## CHARACTERIZATION OF LEWY BODY PATHOLOGY IN 12- AND 16 YEAR OLD INTRASTRIATAL MESENCEPHALIC GRAFTS SURVIVING IN A PATIENT WITH PARKINSON'S DISEASE

**Jia-Yi Li**<sup>1</sup>, Elisabet Englund<sup>2</sup>, Håkan Widner<sup>3</sup>, Stig Rehncrona<sup>4</sup>, Anders Björklund<sup>5</sup>, Olle Lindvall<sup>3,6,#</sup> and Patrik Brundin<sup>1,#</sup>

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We previously reported the occurrence of Lewy bodies in grafted human fetal mesencephalic neurons in two patients with Parkinson's disease. Here we have used immunohistochemistry and electron microscopy to characterize the development of Lewy bodies in one of these cases. This patient was operated in putamen on both sides at 12 or 16 years before death, respectively. We demonstrate that 2% of the 12-year old and 5% of the 16-year old grafted, presumed dopaminergic neurons contained Lewy bodies immunoreactive for  $\alpha$ -synuclein. Based on morphological analysis, two forms of  $\alpha$ -synuclein-positive aggregates were distinguished in the grafts, the first a classical and compact Lewy body, the other a loose meshwork aggregate. Lewy bodies in the grafts stained positively for ubiquitin and thioflavin-S, and contained characteristic  $\alpha$ -synuclein immunoreactive fibrillar structures on electron microscopy. Our data indicate that Lewy bodies develop gradually in transplanted dopaminergic neurons in a fashion similar to that in dopaminergic neurons in the host substantia nigra.

## HUMAN-DERIVED VENTRAL MESENCEPHALIC DOPAMINERGIC GRAFTS IN THE 6-OHDA RAT MODEL OF PARKINSON'S DISEASE

**Anika Rath**, Alexander Klein, Anna Papazoglou, Joanna Garcia and Guido Nikkhah

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**Background:** Cell-restorative therapy in patients with PD by transplantation of VM DA grafts is a highly promising prospect for future clinical treatment.

**Aims:** The aim of this study was to compare different transplantation strategies and grafting locations for the transplantation of human-derived VM grafts into the 6-OHDA rat model of PD. Graft survival and functional recovery were evaluated.

**Methods:** Unilateral injections of 6-OHDA into the MFB of rats led to a depletion of dopamine within the relevant striatum. Four weeks after the lesion, foetal (6-12 weeks) human VM cell suspensions were transplanted by using different techniques and target locations: group 1 and 2 received a single cell suspension transplanted by glass capillary into the nigra and into the striatum respectively by dividing one VM in two. Group 3 and 4 were transplanted by metal cannula into the striatum and received small tissue pieces and a single cell suspension respectively by dividing the VMs. Each animal received a total of  $4 \times 10^5$  cells. Drug-induced rotation tests were performed 3, 6, 10 and 14 weeks after the transplantation. Immunosuppression was succeeded by daily injections of Cyclosporin.

**Results:** Groups 2, 3 and 4 showed a significant reduction in the amphetamine-induced rotation tests after 10 and 14 weeks, whereas no changes were seen in group 1. All groups showed a clear reduction in the apomorphine-induced rotation test after 6 weeks. The survival of DAergic cells as well as the graft volume were significantly higher in group 3 than in group 2 and 4.

**Conclusions:** Human VM cells transplanted in the 6-OHDA rat model survived well up to 14 weeks. The reduction in the amphetamine-induced rotation tests in all groups transplanted into the striatum shows a functional recovery, whereas human grafts in the nigra do not seem to increase the disposable dopamine in the striatum. The transplantation of human tissue pieces by metal cannula seems to be more favourable to the transplantation of a single cell suspension by cannula, demonstrated by a stronger reduction in the rotation tests and a better cell survival. Taken together our study provides useful and important information for the further methodological refinements of neural transplantation for PD.

**SATURDAY 28<sup>TH</sup> NOVEMBER****SESSION 6****STEMSTROKE****EU sponsored FP6 STEMSTROKE symposium**

The Symposium-Round Table is devoted to the current status and needs for stroke therapy, and the particular opportunities offered by stem cells for development of new therapeutic strategies. Leading stroke clinicians and stem cell researchers will discuss how to translate current knowledge in stroke and basic stem cell research and what needs to be done in clinic in order to develop stem cell therapy for stroke patients and is open to all NECTAR delegates.

Chairman: Prof. Olle Lindvall, StemStroke's Chief Clinical Advisor,  
Lund University Hospital, Sweden

**Overview session**

08:30-08:45	Bo Norrving	Welcome and Introduction
08:45-09:15	Stephen Meairs	Challenges for stroke therapy
09:15-10:00	Summary of achievements from clinical perspectives	
	1. <b>STEMSTROKE</b> - Zaal Kokaia	
	2. <b>STEMS</b> - Brigitte Onteniente	
10:00-10:40	Geoffrey Donnan	Clinical translation of stem cells in stroke
<b>10:40-11:00</b>	<b>Coffee break</b>	

**Round Table**

11:00-11:50	Contributors and panel members:	
	Mathias Hoehn	Imaging for stem cell therapy
	Stephen Dunnett	Behavioral test in animal models and patients
	Arne Lindgren	Stroke patient selection for stem cell therapy
	Bo Norrving	
	Geoffrey Donnan	
	Zaal Kokaia	
	Stephen Meairs	
11:50-12:00	Olle Lindvall	Closing remarks
<b>12:00 – 13:00</b>	<b>Lunch</b>	



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