

# Abstracts from the 12th meeting of the International Society for Neural Transplantation and Restoration

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## Note from the Publisher

The following abstracts section presents abstracts from all presenters at the 12th meeting of the International Society for Neural Transplantation and Restoration.

They were meant to be included in last year's December issue of NeuroReport, but unfortunately were omitted. The Publisher and Editors apologize to readers and provide them here in the February 2014 issue.

## ABSTRACTS OF ORAL PRESENTATIONS

### Repairing neural circuits with local circuit neurons

Arturo Alvarez-Buylla  
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Cortical GABAergic interneurons originate in the subpial ganglionic eminences (GE). Their integration into the forebrain and cortical circuits is essential to achieve proper inhibitory-excitatory balance and appears to also be key for the induction of cortical plasticity. Importantly, these cells retain their long-range migratory potential and can integrate into neural circuits when grafted into the postnatal and adult brain. Remarkably, their numbers are not adjusted by exogenous signals, but internally in a cell or population autonomous manner. This allows for the addition by transplantation of numerous interneurons to cortex that disperse and functionally integrate. These grafted cells can reinitiate periods of cortical plasticity and can repair imbalances in animal models of epilepsy. Cortical interneuron transplantation is likely to have a wider therapeutic use as we learn more about how specific subtypes of these GABAergic cells are affected in different human diseases. I will discuss recent data suggesting the large-scale contribution of caudal and probably lateral GEs to the human cortical interneuron population. Interestingly, some of these large streams of putative migrating interneurons are retained into the postnatal human brain. These findings suggest new approaches for the repair of cortical circuits.

### Directly reprogrammed neural precursors from patient-specific fibroblasts

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Yamanaka *et al.* 2007, appeared as a mile stone in cell reprogramming exploring a source of induced pluripotent

stem (iPS) cells, a valuable tool and ally for cell therapy. Nevertheless, the process continued to be complicated and time consumption, involving risks of vector integrations. We combined an insertion free approach of transfecting 3 plasmids carrying the reprogramming factors (Okita *et al.*, 2011) into fibroblasts from Huntington's disease (HD) patients.

The cells were propagated under neuralizing conditions and neural rosettes (2 weeks after transfection) were allowed to get confluent, picked and expanded; leading to a high yield of directly reprogrammed neural cells (drNCs).

drNCs were positive for Nestin, BIII Tubulin, PLZF and ZO1; and evidenced Neurotensin, FoxG1, LHX9 and Pax6. Thereafter the exposure to FGF2, FGF8, Purmorphine and CHIR99020; DLX, DSH, OTX2, GAD67 and Darpp32 were confirmed.

21 weeks after transplantation, Nestin, BIII, PSA-NCAM, GFAP, Oligodendrocyte2 and Glutamate were positive with no signs of tumor formation.

This model, gave rise an early developmental phenotype of stem cells (forebrain). It evidenced malleability through addition of growth factors and small molecules: rostralization of an established commitment, including Darpp32 positive cells. It highlights a valuable tool in understanding the neurodegenerative diseases and neural development.

### Neurotrophins: from bench to bedside

Yves-Alain Barde  
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Neurotrophins are small dimeric proteins encoded by 4 genes in mouse and human. The lack of such genes in species used by geneticists to dissect signaling pathways has seriously complicated progress in this field. Thus, only recently was it realized that 2 of the long known neurotrophin tyrosine kinase receptors, TrkA and TrkC actually kill neurons in the absence of ligand-mediated activation, thus explaining the dependency for survival of some neurons on nerve growth factor established over 50 years ago [1]. In addition, the strong structural relatedness of neurotrophins and their receptors led to unwarranted extrapolations about their mode of action. In particular, nerve growth factor in the peripheral nervous system served as the dominant conceptual framework to infer how neurotrophins would work in the brain. But recent results indicate that brain-derived neurotrophic factor (BDNF), by far the most widespread neurotrophin in the brain, is localized pre- and not post-synaptically in the nerve terminals of excitatory neurons [2] and that it is not a

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significant survival factor for most CNS neurons [3]. This is in line with functional results obtained by others indicating that the rodent striatum is fed forward by cortical afferents delivering BDNF, a significant body of work of high relevance to diseases such as Huntington's. This feed forward mechanism seems to be needed by many GABA-ergic neurons to reach, possibly also to keep, their normal size even if BDNF is *not* needed for their survival. For example, in animals lacking *Mecp2*, the gene associated with most cases of Rett Syndrome, the levels of BDNF are markedly reduced in some brain areas, including the cerebral cortex. In young adult animals, the size of the striatum is significantly decreased in *Mecp2* mutants [4] and we recently showed that the sphingosine analogue fingolimod, a drug recently introduced as the first oral treatment of multiple sclerosis, increases BDNF levels and restores the size of the striatum to a volume close to normal [4]. It is possible then that BDNF levels may modulate the growth of some brain areas even in the adult, a notion that has hardly been explored thus far, in large part because of misleading results obtained with transgenic animals overexpressing BDNF. However, recent results from our laboratory indicate that BDNF overexpression leads to the secretion of unprocessed pro-BDNF, a protein counteracting the growth effects mediated by processed BDNF. By contrast, results obtained with another mouse mutant indicate that increased levels of physiologically processed BDNF are accompanied by a progressive, massive growth of some brain areas in the adult [2,5]. These new results suggest then that while BDNF is not a significant factor for the survival of most CNS neurons, in line with finding that its receptor TrkB does not cause neuronal death in the absence of ligand [1], it may regulate the size of various brain areas, even in the adult.

## References

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## TRANSEURO: what has it achieved to date?

Roger A. Barker  
Cambridge, UK

Trials of human fetal ventral mesencephalic tissue (hfVM) transplants in patients with Parkinson's Disease (PD) have produced mixed results with some individuals showing

excellent long term outcomes and others developing side-effects without benefit. The reasons for this may relate to patient selection, tissue preparation and support (pre and post implantation) and trial design. In 2006 a working group was set up to discuss these issues which led to the successful funding of an EU-FP7 grant looking at hfVM grafts in a new trial, called TRANSEURO. Over the last 3–4 years TRANSEURO has moved to the point of starting this new trial by addressing issues in the laboratory to do with tissue preparation at a GMP level, as well as recruiting and following up a cohort of younger early stage patients using clinical and imaging assessments. In this talk I will outline the rationale and status of this new trial.

## Stem cell therapy for stroke: preclinical evidence and an update on FDA-approved clinical trials

Cesar V. Borlongan  
Tampa, Florida, USA

*Background and purpose:* Stem cells possess excellent therapeutic applications for ischemic brain injury. In this talk, I will provide scientific evidence that intravascular and intracerebral transplantation of stem cells produce behavioral and histological benefits in experimental models of stroke. I will highlight gating preclinical evidence that form the basis for translating stem cell therapy in stroke from the laboratory to the clinic.

*Methods:* In carefully assessing the safety and efficacy of stem cell therapy for stroke, I refer to the 3 meetings of STEPS or Stem cell Therapeutics as an Emerging Paradigm for Stroke. Here, I will discuss the lab-to-clinic experimental design that is of keen interest to the academicians, physicians, NIH program directors, US FDA regulators, and biomedical company stakeholders.

*Results:* To date, there are 9 FDA-approved clinical trials of stem cell therapy for stroke. Safety and efficacy outcomes from these trials remain to be reported.

*Conclusion:* Stem cell therapy for stroke remains an experimental treatment, and currently being tested in limited clinical trials. The need for transparent translational guidance on how to expedite the entry of this cell therapy to the clinic is warranted to abrogate the mortality and morbidity inherent in stroke.

*Disclaimer:* CVB holds patents and patent applications related to stem cell therapy, and serves as consultant and editor to a number of stem cell-related biotech companies and scientific journals. Funded by NIH, Celgene, and Sanbio.

## Cellular autonomy and neurodegeneration

Siddharthan Chandran  
Edinburgh, UK

Neurodegenerative disorders such as Alzheimer's disease, amyotrophic lateral sclerosis (ALS) and Parkinson's disease are characterised by the selective loss of specific neuronal

subtypes. While the precise mechanisms underlying neuronal dysfunction and subtype specificity remain the subject of intense study, the cellular environment in which degenerating neurons reside has been shown to be an integral part of the disease. In turn, until comparatively recently glial damage and death has been the focus of other diseases such as multiple sclerosis. An emerging consensus across these diseases is that, dependent on context, glia can be injurious - setting the pace of, and perhaps in some instances initiating neurodegeneration - or even neuroprotective. My talk will discuss glial-neuronal interaction and the opportunities human stem cell systems offer to model aspects of cellular autonomy in the context of in the context of neurodegeneration - regeneration.

### **AAV9-mediated EPO gene delivery in a rat model of Parkinson's disease**

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The aims of the work were to examine cellular and behavioral effects of intrastriatal injections of adeno-associated virus serotype 9 vectors - the human EPO gene (AAV9-hEPO) into the brain of 6-OHDA-lesioned rats, and to evaluate inflammatory and immune responses against AAV9-hEPO vectors in the striatum of the rats. We observed that expression of the human EPO gene was robust and stable in the striatum and the substantia nigra. As a result, nigral dopaminergic neurons were protected against 6-OHDA-induced toxicity. Amphetamine-induced rotational asymmetry and spontaneous forelimb use asymmetry were both attenuated. Intramuscular, but not intrastriatal injections of AAV9-hEPO resulted in reduced levels of hEPO transduction and increased levels of the major histocompatibility complex class I and class II antigen expression in the striatum following AAV9-hEPO re-administration. There were infiltration of the cluster of differentiation 4 (CD4)- and CD8-lymphocytes, and accumulation of activated microglial cells and astrocytes in the virally injected striatum. In addition, the sera from the rats with intramuscular injections of AAV9-hEPO contained greater levels of antibodies against both AAV9 capsid protein and hEPO protein than the other treatment groups.

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### **"The Land of My Fathers": the pioneers of neural transplantation in whose footsteps we walk**

Stephen B. Dunnett

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The Welsh national anthem reminds us that it is good to know the roots from where we come, and inspires us to

build upon the past struggles of the forefathers of our field that have allowed us to get to where we are today.

I remember the early attempts at cell transplantation from Paré, Thompson and Dunn to the consistent achievements of Gleees and Le Gros Clark. I consider the widespread neglect of the quite remarkable results of these pioneers by the biologists of the day in the light of an (over-rigid) interpretation of Cajal's dictum that all regeneration in the adult mammalian central nervous system is invariably abortive. That *zeitgeist* was only convincingly challenged with the synchronous studies of Raisman, Altman and Olson at the turn of the 1970s using novel anatomical techniques for ultrastructural pathway tracing, thymidine birthdating, and intraocular transplantation, respectively. Using the new methods of catecholamine fluorescence to visualise specific populations of central and peripheral neurons and their connections, two schools rapidly emerged in central and southern Sweden under the leaderships and inspiration of Lars Olson at the Karolinska and Anders Björklund in Lund, respectively, that defined the conditions for cell transplantation in the adult mammalian brain. The first wave of studies using these new techniques sought to characterise the principles of cell survival, axon growth, targeting and formation of appropriate connections in development and in regeneration using primarily anatomical tools.

Then at the turn of the 1980s, a second wave of activity broadened the field to investigate the extent to which transplanted cells could have a functional impact on the behaviours of the host animal, opening the way to development of reparative cell based therapies. A first set of pioneering studies demonstrated functional recovery of motor symptoms in rats with dopamine-depleting lesions following embryonic mesencephalic transplants to effect dopamine-cell replacement, and led swiftly (some might think excessively so) to the first clinical trials in patients of adrenal cell transplants. These observations stimulated a proliferation of applications in other model systems, which I illustrate by the studies of (i) John Sladek and colleagues on neuroendocrine function by hypothalamic repair in the *diabetes insipidus* Brattleboro rat; (ii) Albert Aguayo and the McGill team on peripheral nerve bridges to allow long distance central axon growth across the site of injury in spinal cord; and (iii) Ray Lund and team on restoration of retinal tectal reconnections. These latter studies included the first indication that rehabilitation is essential in conjunction with structural repair to maximise functional recovery, and provide the foundations for the first in man clinical trials of pluripotent and stem cell transplantation in macular degeneration, presented elsewhere in this international symposium. These foundations underpin the explosion of use of transplantation technologies over the last two decades both for the analysis of fundamental neurobiology of brain development, plasticity and reorganisation, as well as providing the basis for the rapid growth of cell therapy as a leading strategy in contemporary regenerative medicine.

Finally, I remember the history of the INTR symposia themselves, the first of which was held in Lund in 1984 and which have continued on a regular 2-3 year cycle ever since. The INTR symposia have established and continue to foster a rare spirit of collegiality and collaboration in our field of neural transplantation research, as well as having spawned the regional societies of NECTAR and ASNTR in Europe and North America respectively. We look forward with great optimism to the 13<sup>th</sup> International Symposium to be held in Beijing in 2013.

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## Targeting the extracellular matrix to repair the damaged nervous system

James W. Fawcett  
Cambridge, UK

Repair of damage to the nervous system requires axon regeneration, plasticity and cell replacement. The extracellular matrix plays a central role in restricting these processes, mainly through the action of chondroitin sulphate proteoglycans (CSPGs). An important agent in removing this restriction has been the enzyme chondroitinase, which removes glycosaminoglycan (GAG) chains from CSPGs. CSPGs are up-regulated in glial scar tissue around injuries to restrict axon regeneration, which can be enhanced with chondroitinase. However the positive effect of chondroitinase on functional recovery is mostly through reactivation of plasticity. Chondroitinase treatment enhances recovery from many forms of CNS damage, including spinal cord injury, where the reactivation of plasticity enables successful rehabilitation. Chondroitinase also has profound effects on memory, prolonging object memory in normal animals and restoring memory in an Alzheimer's model. CSPGs control plasticity mainly through their participation in perineuronal nets (PNNs), cartilage-like structures surrounding neurons, which appear as critical periods for plasticity close. PNNs contain inhibitory CSPGs, hyaluronan, link protein and tenascin-R, partly produced by the neurones themselves and partly by surrounding glial cells. All neurones with PNNs express both a hyaluronan synthase enzyme and a link protein, and these are the key components that trigger the formation of the structures. Link protein knockout animals lack normal PNNs on their dendrites, and these animals retain plasticity into adulthood, and show prolongation of memory identically to animals treated with chondroitinase. The action of the CSPGs is due to their sulphated GAGs. In the CNS these bind to and localise Semaphorin3A and OTX2 to PNNs. OTX2 is involved in the maturation of inhibitory interneurons, while Semaphorin3A is an effector of the PNNs involved in control of plasticity.

## Grafting of rat and porcine fetal neuronal cells into the subthalamic nucleus in experimental epilepsy

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Neuronal transplantation into brain regions involved in seizure generation or propagation is a promising experimental approach to treat drug-resistant epilepsies. We recently showed that the subthalamic nucleus (STN) might be a promising target region in this respect (Bröer *et al.* 2012, *Neurobiol Dis.* 46: 362–376). We now tested two types of rat and porcine fetal stem cells (RNSCs and PNSCs) isolated from: (A) medial ganglionic eminence (MGE), and (B) mesencephalon. Grafts of both cell types cultivated as neurospheres were inserted bilaterally into the STN (80 000 cells per side). Anticonvulsant efficacy was evaluated by testing seizure thresholds before and at different time-points after grafting (from 10 days up to 3 months).

RNCSs and PNSCs derived from the mesencephalon, but not from the MGE, showed clear anticonvulsant effects ten days and moderate effects 3 months after grafting. PNSCs differentiated into neurons and astrocytes and showed expression of the GABA-synthesizing enzyme glutamic acid decarboxylase. These data indicate that grafting of neuronal mesencephalic stem cells into the STN could be a promising approach to treat epilepsy. Future work will focus on improving duration of anticonvulsant efficacy after grafting into the STN.

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## GDNF – Back in the clinic

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*Objective:* To evaluate in PD the efficacy and safety of intermittent GDNF intra-putaminal infusions administered by Convection Enhanced Delivery.

*Background:* In animal models of PD and in open label studies in PD patients, continuous intra-putaminal GDNF infusions have been shown to improve motor symptoms and, as assessed by 18F-dopa PET and post-mortem assessments, restore dopamine terminals. However, in 2003, a placebo-controlled multi-centre trial reported failure to demonstrate clinical benefit, despite improvements in PET end-points. In addition, a concurrent study in monkeys raised questions over safety. Since that point no further human investigations have been performed. In significant part, we believe, the above issues were due to a failure in the way GDNF was surgically delivered. We have now developed an in-house device which animal model studies suggest will allow GDNF to be given much more

reliably to the putamen. We feel this allows for definitive testing of GDNF's effects in humans.

**Methods:** A phase II, single centre, randomized, double blind, placebo-controlled trial, in idiopathic PD ( $n = 42$ ), of intermittent bilateral posterior putamen GDNF infusions administered via Convection Enhanced Delivery (CED) was commenced in 2012. The drug delivery system comprises 4 micro-catheters, with in-line filters, and a skull mounted transcutaneous drug delivery port with 4 independent channels. 4 programmable pumps are connected to the port via an administration set on a 2 or 4 weekly basis for intermittent infusions of GDNF or placebo (aCSF) through the course of this trial.

**Results:** The design of this 3 phase trial: Pilot Stage; Primary Study stage; and Roll-Over Stage will be discussed as well as describing the technological challenges of achieving reliable intermittent CED of GDNF over the long term and how we are attempting to address these.

**Conclusion:** This will be the first clinical study of intermittent GDNF infusions delivered by CED in man.

#### **Generation of human neural progenitor cells from induced pluripotent stem cells that survive, migrate and integrate into the rodent spinal cord**

Geneviève Gowing, Dhruv Sareen, Anais Sahabian, Kevin Staggenborg, Renée Paradis, Pablo Avalos, Jessica Latter, Loren Ornelas, Leslie Garcia and Clive N. Svendsen  
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Transplantation of human neural progenitors (hNPC) derived from pluripotent stem cells (PSC's) is a promising therapeutic strategy that has the potential to replace lost cells, modulate the injury environment and create a permissive milieu for the protection or regeneration of host neurons in disease. Here we use a novel chopping technique to isolate and expand EZ spheres from PSC's which were then driven to an expandable iNPC spinal cord phenotype using a combination of retinoic acid followed by the mitogens EGF and FGF-2. iNPCs grown in suspension showed similar characteristics to hNPCs derived from human fetal tissues, although iNPCs grown as an adherent culture did not. Suspension iNPCs were easy to maintain using the chopping method of expansion and survived grafting into the spinal cord of athymic nude rats with no signs of overgrowth, again with very similar profiles to hNPCs derived from fetal tissues. These results suggest that iPSC-derived NPC's could be a favorable alternative to fetal hNPCs for cellular regenerative therapies of CNS diseases.

#### **Modulating endogenous stem cells to restore learning and memory in Temporal Lobe Epilepsy**

William P. Gray  
*Cardiff, Wales, UK*

Neurogenesis, the production of new neurons, is a restricted event in the adult human brain, largely confined to the dentate gyrus where it is important for hippocampal learning and memory. While early studies showed conflicting roles for neurogenesis in spatial learning & memory, more recent work using paradigms that involve a higher cognitive demand, have demonstrated roles for neurogenesis in both the acquisition and retrieval of spatial relational memory. Altered neurogenesis also appears to play a role in anxiety and depression. Interestingly antidepressants increase hippocampal neurogenesis, an effect that appears necessary for their behavioural effects on mood and anxiety.

Hippocampal neurogenesis is affected by a myriad of factors from neural activity (learning), exercise, drugs, metabolism, the immune system as well as brain injury and disease. Altered neurogenesis may thus be an important mechanism, underlying cognitive dysfunction and altered mood across a wide variety of neurological diseases, and as such presents an attractive target for medical and surgical interventions. I will present an overview of hippocampal neurogenesis, followed by an analysis of how it is affected by temporal lobe epilepsy, and emerging strategies from our lab and others, on how it may be restored.

#### **The origins and future of pluripotency and cellular reprogramming**

John B. Gurdon  
*Cambridge, UK*

The different cell types that compose our bodies are remarkably stable. Hardly ever do we find skin cells in the brain or liver cells in the heart. In those very special cases where some regeneration can take place in vertebrates, there is little if any evidence for a switch in cell-type. Nevertheless, nuclear transfer, cell fusion, and induced pluripotency can result in pluripotent embryo cells being derived from specialized adult cells. The mechanisms by which nuclear reprogramming can occur in these cases is beginning to be understood. It may become possible for new, regenerated cell-types to be derived from adult cells and given back to a patient so that they receive new cells of their own genetic constitution, thereby avoiding the need for immunosuppression. The history of work in this area, and the prospects for cell replacement in the future will be discussed.

#### **Imaging stem cell fate following intracerebral grafting**

Philippe Hantraye  
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When paving the way towards clinical applications of stem cell grafting, one crucial step is the ability to ascertain whether once implanted in a particular individual the pre-differentiated stem cells are actually evolving normally. In fact, a longitudinal, non-invasive follow-up should help us predict whether cells giving rise

to the expected neuronal cell type or, on the contrary, turning into an unnecessary mixture of neurons and/or glial cells or even leading to an uncontrolled growth of cells that would potentially be harmful to the patient.

In vivo imaging may be helpful in solving these questions provided that each of the available modalities has been carefully validated and tested for its capacity to differentiate the normal and/or the pathological fate of the cells following grafting in a living being. In addition, as these techniques have to be applied to a clinical setting, their use should meet strict regulatory and safety criteria.

Of all available techniques, magnetic resonance imaging (MRI) has been long used to obtain information about graft placement, the in vivo monitoring of graft growth as well as occurrence of abnormal phenomena either associated with the grafting procedure itself (mechanical stress, hemorrhages) or the potential death of the grafted cells occurring shortly following their implantation (oedema). Besides MRI, alternative MR techniques have also been developed and studied for their potential use in stem cell research to precise the fate of these cells in the living being. In this context, <sup>1</sup>H-MR spectroscopy has proven capable of providing a biochemical signature of the graft composition in vivo, which helps differentiate a graft presenting with the expected proportion of neurons and another, undoubtedly enriched in astrocytes or other glial cell types. In addition, diffusion-weighted MR imaging may also yield invaluable information on how the grafted cells extend significant processes out of their implantation sites and efficiently reconnect remote regions of the host's brain in a normal or unexpected way.

Most certainly, one of the imaging techniques that holds most promise in medical applications of stem cell research is positron emission tomography. This radioisotopic technique enables one to map and quantify the concentrations of any molecule previously labeled with a positron-emitting isotope. Depending on the radiotracer being selected for the study, PET can either serve as a means to detect adverse effects like neuroinflammation and cell rejection or to study the phenotypic differentiation of the graft using markers that are specific of the expected neuronal cell population. Metabolic markers like <sup>18</sup>F-fluorodeoxyglucose can also help differentiate between surviving neurons and dying cells. Reuptake blockers like the cocaine analog <sup>18</sup>F-LBT999 can also evidence the regrowth of presynaptic terminals, innervating *de novo* the grafted stem cells neo-differentiated into striatal GABAergic neurons. Very similarly, specific radiomarkers of the pre- and post-synaptic elements of the dopaminergic synapse can also be used to establish the functional status and the degree of integration of stem cells programmed to differentiate into a dopaminergic phenotype.

When preparing a clinical trial, it is certain that a combination rather than the sole use of one of these imaging approaches has to be envisioned. Preclinical trials have also shown that the best combination of imaging

techniques/markers may vary greatly in function of stem cell type to be used.

Examples of the use of these various imaging techniques will be provided and their advantages and disadvantages will be discussed in the context of the preclinical application of stem cells for Huntington's and Parkinson's diseases.

### **Global clinical neurorestoration in complete chronic spinal cord injury**

Hongyun Huang, Tiansheng Sun, Lin Chen, Milan Dimitrijevic, Gustavo Maviglia, Elena Chernykh, Klaus von Wild, Haluk Deda, Kyung-Sun Kang, Anand Kumar, Sang Ryong Jeon, Shaocheng Zhang, Giorgio Brunelli, Albert Bohbot, Maria Dolors, Jianjun Li, Alexandre Fogaça Cristante, Haitao Xi, Gelu Onose, Helmut Kern, Ugo Carraro, Hooshang Saberi, Hari Shanker Sharma, Alok Sharma, Xijing He, Dafin Muresanu, Shiqing Feng, Ali Otom, Dajue Wang, Koichi Iwatsu and (all authors from 19 countries)  
*Beijing, China*

It is still popular in medical community that there are no any effective therapeutic methods to restore neurological functions for lesion. Though the treatment to recover function in people suffering complete spinal cord injury (SCI) remains a big challenge for clinical physicians, accumulating data have shown that partial clinical neurorestoration is possible by using cell therapy, neurostimulation or neuromodulation, neuroprosthesis or related advanced assistive devices, neurotization or nerve bridging, neurorehabilitation. Here we summarize the literatures that demonstrate patients with chronic complete SCI have been able to obtain some clinical neurorestoration, which is based on the scientific information available till April of 2013, and we discuss several important issues about these evidences. The goal of this review is to show the objective "yes or no" evidences of clinical neurorestoration for chronic complete SCI, which will make more people know real progresses in this field. And we hope the medical community is able to highlight the value of clinical neurorestorative treatments for CNS incurable diseases.

### **Very long-term clinical outcome of fetal cell transplantation for Parkinson's disease**

Zinovia Kefalopoulou<sup>a</sup>, Marios Politis<sup>a</sup>, Paola Piccini<sup>a</sup>, Nicolo Mencacci<sup>a</sup>, Kailash Bhatia<sup>a</sup>, Marjan Jahanshahi<sup>a</sup>, Håkan Widner<sup>b</sup>, Stig Rehncrona<sup>b</sup>, Patrik Brundin<sup>b,c</sup>, Anders Björklund<sup>b</sup>, Olle Lindvall<sup>b</sup>, Patricia Limousin<sup>a</sup>, Niall Quinn<sup>a</sup> and Thomas Foltynie<sup>a</sup>  
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Recent advances in stem cell technologies have renewed an interest in the use of cell replacement strategies for patients with Parkinson's disease (PD). This study reports the very long-term clinical outcomes of fetal cell transplantation in two patients with PD. Such long-term follow-up data can

usefully inform on the potential efficacy of this approach, as well as the design of trials for its further evaluation.

Two patients received intrastriatal grafts of human fetal ventral mesencephalic tissue, rich in dopaminergic neuroblasts, as restorative treatment for their PD. To evaluate the long-term efficacy of the grafts, clinical assessments were performed 18 and 15 years post-transplantation. Motor improvements developed and increased gradually over the first post-operative years, and are sustained up to 18 years post-transplantation. Despite each patient having nearly 30-years of PD with troublesome fluctuations and dyskinesias prior to transplantation, both patients now present with mild symptoms, and are independent of any pharmacological dopaminergic treatment for more than 10 years. Both patients have developed mild/moderate graft-induced dyskinesias.

The results from these two cases indicate that dopaminergic cell transplantation can offer very long-term symptomatic relief in carefully selected PD patients, and provide proof-of-concept support for future clinical trials using fetal or stem cell therapies.

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#### **Anti-amyloid precursor protein antibodies as a potential therapy for Alzheimer's disease**

Emma J. Kidd, Martha Hvoslef-Eide and Rhian S. Thomas  
*Cardiff, UK*

Cleavage of amyloid precursor protein (APP) by  $\beta$ - and  $\gamma$ -secretases results in amyloid- $\beta$  (A $\beta$ ) production in Alzheimer's disease. We raised two antibodies to the  $\beta$ -secretase cleavage site in APP. Our hypothesis was that these antibodies would reduce A $\beta$  levels via steric hindrance of  $\beta$ -secretase.

Cells were incubated with various antibodies and APP, its cleavage fragments and the affinities of the antibodies for binding to the cleavage site and likely epitopes were measured by ELISAs. The ability of antibodies to enter cells was examined by immunocytochemistry. Block of  $\beta$ -secretase by steric hindrance was investigated.

Both antibodies significantly reduced A $\beta$  levels with 2B3 being more effective than 2B12. 2B3 had higher affinity for the  $\beta$ -secretase cleavage site than 2B12 and bound across the cleavage site while 2B12 bound upstream. Both antibodies entered living cells and inhibited the action of  $\beta$ -secretase.

2B3 is a more potent antibody than 2B12 to reduce A $\beta$  levels in agreement with its higher affinity for the cleavage site and epitope location. We have proved our hypothesis that these antibodies block the  $\beta$ -secretase cleavage site. 2B3 is being considered as a therapeutic antibody for Alzheimer's disease.

*Acknowledgements:* This work was funded by the Alzheimer's Society.

#### **Human ventral mesencephalic grafts alleviate both motor and non-motor dysfunctions in a rodent model of PD**

Mariah J. Lelos, Claire M. Kelly, Eduardo M. Torres, Anne E. Rosser and Stephen B. Dunnett  
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Although it remains the current 'gold standard' cell replacement therapy, characterisation of the functional efficacy of human primary tissue is currently limited to the alleviation of simple motor deficits. Given that Parkinson's disease patients also develop impairments in a range of non-motor domains, including neuropsychiatric and cognitive deficits, it is critical to evaluate the cell therapies in terms of their impact upon these non-motor dysfunctions. To address this, we pre-trained rats on a lateralised reaction-time task, before they received a unilateral 6-OHDA MFB lesion. A subset of lesion rats were then grafted with suspensions of human ventral mesencephalic tissue (hVM), of ~9 weeks gestation. On both amphetamine- and apomorphine-induced rotation tests, rats grafted with hVM displayed a significant reduction in rotational bias. Twelve weeks post-graft, rats were tested on two distinct versions of the operant task. Results from grafted rats revealed enhanced motor function (movement time, reaction time) as well as significant improvement of non-motor impairments, including motivational and spatial processing (total trials completed, accuracy). These data are the first to demonstrate that VM tissue from the human fetus is capable of alleviating the non-motor dysfunctions induced by loss of striatal dopamine in Parkinson's disease.

*Acknowledgements:* Funded by FP7 NeuroStemCell.

#### **Directing striatal GABAergic fate specification of human pluripotent stem cells**

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The  $\gamma$ -amino butyric acid (GABA) medium-sized spiny neurons (MSNs) are the principal projection neurons of the striatum, which specifically degenerate in the early phase of Huntington's disease. Unlike some other neurodegenerative diseases, such as Parkinson's, which can be effectively managed for years by dopamine-based pharmacotherapy, no effective treatment is currently available for Huntington's. MSNs derived from human pluripotent stem cells (hPSCs) promise hope for developing transplantation-based cell therapy, studying disease aetiology and drug screening for HD. We discovered that the TGF $\beta$  molecule activin can induce lateral GE (LGE)/striatal characteristics in forebrain

neural progenitors derived from hESCs and hiPSCs. These progenitors readily differentiate into post-mitotic neurons expressing the signature marker of MSNs, dopamine- and cAMP-regulated neuronal phosphoprotein (DARPP32) in culture, as well as following transplantation into the striatum of a rat model of Huntington's disease. Significantly, grafts derived from activin A-induced striatal progenitors showed no cellular expansion and were tumour-free 16 weeks post-transplantation. Together, our findings demonstrate a novel route for directed differentiation of transplantable MSNs from hPSCs.

### **Gene therapies for hereditary Parkinson's disease; a strategic transition from *in vivo* to *ex vivo***

Hideki Mochizuki

Osaka, Japan

In mouse and monkey models of Parkinson's disease (PD), we have previously shown that intracerebral injection of adeno-associated viral vector encoding parkin, which is the causative gene of autosomal-recessive juvenile-onset PD *PARK2*, resulted in rescue of neuronal and behavioral phenotypes [1,2]. Delivery of parkin counteracted the attenuation of PKB/Akt pathway and protected dopaminergic neurons from apoptotic death in PD mice [2]. However, the proportion of neuronal survival was limited to a small extent and mitochondrial abnormality was not corrected in the *in vivo* gene therapy. Meanwhile, a recent advances on iPS cell technology led us to establishment of a novel *ex vivo* gene therapy for hereditary PD. We have generated dopaminergic neurons *in vitro* from iPS cells, derived from *PARK2* patients, and found mitochondrial dysfunction and accumulation of alpha-synuclein, which resembled the histopathology in a donor patient [3]. We are studying the effects of transduction of wild-type parkin to the parkin-deficient iPS cell-derived dopaminergic neurons and transplantation of these cells in mouse and monkey models of PD to evaluate the novel *ex vivo* gene therapy.

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### **Clinical application of retinal pigment epithelium (RPE) cells derived from human embryonic stem cells**

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The retina is part of the central nervous system and contains specialized neurons, photoreceptors, that convert light signals into electric signals, further transmitted to the brain by different neurons. *In vivo*, the retinal pigmented epithelium (RPE) constitutes a distinct monolayer of pigmented cells lying between the neural retina and Bruch's membrane, which provides essential support for the long-term preservation of retinal integrity and visual function. Given the intimate anatomical and functional relationship of RPE cells and photoreceptors, it is not surprising that the first manifestations of primary RPE disorders are problems with visions. Such is the case with age-related macular degeneration (AMD) and some forms of retinitis pigmentosa (RP) caused by mutations in genes involved in central RPE functions. RPE replacement therapies using RPE cells generated from human embryonic stem cells (hESC) provide a novel approach to a rational treatment of such forms of blindness. The aims of our study is to (i) generate a clinically-compatible polarized monolayer of RPE cells derived from human embryonic stem cells (hESC) disposed on a biocompatible membrane and (ii) test their functional properties in RP animal models in order to develop a clinical trial for patients suffering from genetic RPE disorders. The clinical grade hESC line, RC-09 (Roslin Cells, Edinburgh, Scotland) is used for RPE differentiation. Our laboratory already set up and validated a reproducible differentiation protocol to generate pure RPE cells from several human pluripotent stem cells including RC-09. RPE cells obtained are polarized and functional. Our method is robust and productive: a single six-well plate of hESCs can generate  $8 \times 10^7$  RPE cells. This protocol should now be adapted for clinically compatible conditions.

### **Encapsulation of stem cells in an *in situ* gelling collagen hydrogel improves graft volume and striatal retention after intra-cerebral transplantation in the rat**

Ben Newland, Deirdre Hoban, Abhay Pandit and

Eilis Dowd  
Galway, Ireland.

Delivery of neurotrophic factors to the brain via genetically modified mesenchymal stem cells (MSCs) offers a promising neuroprotective strategy for neurodegenerative diseases. However, MSCs delivered to the CNS typically show poor survival post transplantation which limits their therapeutic efficacy. Recent studies have revealed the potential of biomaterials as supportive matrices for transplanted cells which may assist in the grafting process.

In this study, an *in situ* gelling type I collagen hydrogel was evaluated as an intracerebral transplantation matrix for delivery of MSCs to the rat brain. We specifically investigated the effect of the hydrogel on striatal graft volume and retention.



Preliminary *in vitro* studies confirmed that the hydrogel was neither non-toxic to MSCs seeded within it nor to neural cells seeded in juxtaposition to it. We then demonstrated that the collagen hydrogel itself was well tolerated in the rat brain, and was capable of improving the volume of the MSC graft after transplantation. Moreover, the hydrogel completely prevented ectopic deposition of MSCs along the needle track thereby increasing striatal retention of the graft.

These findings indicate the potential of supportive hydrogels for intra-cerebral cell transplantation studies.

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#### **Prosavin, a dopamine gene therapy for advanced PD: phase I clinical update**

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Parkinson's disease (PD) is a neurodegenerative disease that results in a progressive degeneration of dopaminergic neurons. The dopamine precursor L-Dopa and dopamine agonists provide the primary standard of care and demonstrate good therapeutic benefit in the early stages of disease. However, their long term use is associated with severe motor side effects that are at least partially caused by the fluctuating nature of dopaminergic stimulation that arises from oral drug administration. As such, a therapy that provides a more continuous and local supply of dopamine to the site of pathology provides a potential approach for the development of new therapeutic strategies.

ProSavin<sup>®</sup> is a gene therapy product that utilises a lentiviral vector to transfer three genes that are critical for dopamine biosynthesis to the striatum, that is depleted of dopamine in PD.

Clinical evaluation of the safety and efficacy of ProSavin in mid to late stage PD patients is currently ongoing. In the study fifteen patients have received ProSavin<sup>®</sup> in three dose cohorts. ProSavin<sup>®</sup> has been demonstrated to be safe and well tolerated at all doses evaluated to date. There have been no serious adverse events related to ProSavin<sup>®</sup> or the administration procedure and no inflammatory responses. In terms of efficacy an improvement in the primary endpoint, UPDRS Part III, has been observed in all cohorts relative to their baseline scores. Furthermore, an improvement has been maintained out to the latest timepoints evaluated to date (up to 4 years for the earliest cohort). Secondary endpoints including the patients concomitant dopaminergic medications has also demonstrated improvements.

#### **Generating neurons for use in cell therapy; challenges and possibilities**

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Parkinson's Disease (PD) is a particularly interesting target for stem cell based therapy. The central pathology is confined to a small group of neurons in the midbrain, the nigral dopamine (DA) neurons and their projection to the striatum. Transplants of DA neurons could be used to restore DA neurotransmission in the striatum, substitute for the lost neurons, and bring back normal motor behavior. Proof-of-principle that this can work has been obtained in trials where fetal DA neuroblasts, have been transplanted to the putamen in patients with advanced PD. Despite these encouraging results, work with human fetal tissue presents a number of ethical and logistical problems and therefore does not represent a realistic therapeutic option in the future. Further progress in this field is critically dependent on the development of a bankable and renewable source of transplantable DA neurons.

We have developed a method to generate human neural progenitors and neurons from human embryonic stem cells (hESCs), which recapitulates human fetal brain development. By addition of a small molecule to activate canonical WNT signalling, we induced rapid and efficient dose-dependent specification of regionally defined neural progenitors ranging from telencephalic forebrain to posterior hindbrain fates. The DA neurons obtained via our protocol closely resembled their fetal counterparts, making them useful as a model system for studies of human fetal brain development and also for developing transplantable therapeutic cells.

In parallel, we also develop cell reprogramming as an alternative source of neurons. We have found that the neural conversion genes (Mash1, Brn2a, Myt1l) can convert human fibroblasts into induced neurons (iNs). When combined with DA fate determinants, functional DA neurons can be obtained with this technique.

#### **Experimental and clinical transplantation of pluripotent stem cells for cell therapy in retinal disease**

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Several clinical trials of cell based therapy for retinal degeneration are currently recruiting worldwide. This clinical translation is based on over a decade of research into retinal development and physiology. One approach is to generate an appropriate replacement from stem cells, another is to use their paracrine restorative qualities. In tandem, surgical

research into autologous retinal transplantation has evolved techniques to manipulate grafts into the subretinal space. The eye is an ideal organ for an experimental cell based therapy due to its transparency and the immune privilege conferred by the blood retina barrier.

The first trials have focussed on the replacement of the retinal pigment epithelium but ultimately elements of the neural retina must be transplanted to address diseases such as advanced age related macular degeneration and retinitis pigmentosa. Medium and long term follow up of these trials is eagerly awaited.

### **Differentiation of human pluripotent stem cells into brain-regionalized astrocytes to investigate pathogenesis of Parkinson's and Alzheimer's diseases**

Federica Rinaldi, Jennifer Badger, Lucy Crompton and Maeve Caldwell  
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Progressive neuronal degeneration and loss are hallmarks of several neurodegenerative disorders including Parkinson's (PD) and Alzheimer disease (AD); with aetiology and pathophysiology of disease progression not yet fully elucidated. In this study we describe the generation of multipotent neural progenitor (NPs) cells from human stem cells including embryonic (hES) and induced pluripotent (iPS) cells. Our protocol faithfully recapitulates *in vivo* neurogenesis where neurons are generated first followed by astrocytes. We have employed RNA and protein analysis to characterize the transition from pluripotent to NP cells, and to more restricted astroglial progenitors. Ultimately, mimicking morphogen gradients occurring *in vivo* during neural development we have patterned NP cells to acquire basal forebrain and ventral midbrain identity thus successfully deriving brain-regionalized astrocytes. Our results reflect novel data, which have indicated the presence of heterogenic astrocytes distinguished according to their morphology, function, electrical properties and transcriptome. Furthermore our protocol will be of particular interest in the elucidation of the participation of regional astrocytes in neurodegenerative disorders that affect a specific brain region such as PD and AD, previously understood as exclusively neuronal disorders.

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### **Region-specific restoration of striatal synaptic plasticity by dopamine grafts in experimental parkinsonism**

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Maria Angela Cenci<sup>a</sup> and Barbara Picconi<sup>b</sup>  
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Intrastriatal transplantation of dopaminergic neurons can restore dopamine levels and improve parkinsonian

deficits. However, underlying mechanisms are poorly understood. Here we evaluated the synaptic plasticity in the host striatum after neural transplantation using a rat model of Parkinson's disease.

Naïve rats showed distinct synaptic plasticity patterns in striatum. Ventrolateral striatum showed long-term potentiation (LTP) in approximately 63% of the neurons using the same protocol that elicited long-term depression (LTD) in the dorsolateral striatum. The LTP was linked to higher expression of post-synaptic AMPA- and GluN2B NMDA subunits and was shown not to be pathway dependent. In both regions, the synaptic plasticity was abolished after dopamine-depletion and could not be restored by grafted serotonergic neurons. Solely, dopamine grafts restored the LTP and partially restored motor deficits. The restoration was only present in ventrolateral striatum where grafted re-innervation was denser compared to the dorsolateral region.

These data provide a first proof of concept that dopamine transplants are able to functionally integrate into the host brain and restore neuronal deficits in striatal synaptic plasticity after experimental PD. This might have implications for the limitation in symptomatic improvement following transplantation.

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### **Clinical-grade neural progenitor cells secreting GDNF for the treatment of ALS**

Brandon Shelley, Genevieve Gowing, Jessica Latter, Kevin Staggenborg, Pablo Avalos, Leslie Garcia, Renee Paradis, Maximus Chen, Amanda Hurley, Jessica Zelaya and Clive Svendsen  
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Amyotrophic Lateral Sclerosis (ALS) is a disease in which motor neurons die due to an unknown mechanism. Human, fetal cortex-derived neural progenitor cells (hNPCs) can be expanded *in vitro* and survive and integrate into large and small animals following transplantation, such as rat models of ALS. Furthermore, hNPCs can be genetically modified to secrete glial cell line-derived neurotrophic factor (GDNF), a powerful growth factor that has been shown to have a neurotrophic effect in animal models. We have demonstrated that transplanting hNPCs engineered to secrete GDNF can rescue the microenvironment and preserve dying motor neurons in animal models of ALS.

Moving towards the clinic, we have generated a GMP-grade master cell bank of hNPCs and sourced it to generate research and clinical-grade cell lots transduced to secrete GDNF with GMP-grade lentivirus. We have completed the dose ranging aim of the project using an ALS rat model and are moving forward with the safety/

toxicity aim required by the FDA for approval of a phase 1/2a drug safety trial. Here we show the progress made over the first six months of the project and describe future plans, with the eventual goal of transplanting 18 patients.

*Acknowledgements:* CIRM DR2A-05320.

### **From Thompson to Hopkins-Dunn to Ranson; if the Pioneers could see us now!**

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At the first of the series of twelve international meetings on neural transplantation (and now repair) near Lund in 1984, Professors Björklund and Stenevi presented a thoughtful review of the history of our field and drew attention to the findings of early investigators such as Thompson (1890), Faldino (1924), Flerko (1957) and others. At our second meeting (Rochester, 1987) we explored the great progress achieved by our modern pioneers including ongoing clinical experiments and new models of neurological diseases amenable to repair. Clearly W.G. Thompson (NYU) should be credited with the first attempt to graft brain tissue and remarkably in 1909, Walter Ranson (Northwestern Univ.) succeeded in grafting spinal ganglia and observed signs of neuronal plasticity. He described “factors” that must be controlling this cellular transformation, but lamented that there were no known means by which to identify them.

Perhaps I'm biased, but Elizabeth Hopkins Dunn may have made the most important of these early discoveries by grafting comparatively young tissue to the adult brain and at a time when women scientists and physicians were rare and subjected to formidable obstacles to success. After graduation from the Iowa (now Grinnell) College in 1892, Dr. Dunn received her medical degree from the Northwestern University Women's Medical School in 1894 and joined the faculty of the University of Chicago until 1906 when she moved to Woods Hole Marine Biological Laboratories, where her work extended to many other fields including publishing and cartography. On 2 January, 1914 at the 29th annual meeting of the American Association of Anatomists in Cleveland she was the only woman among 13 other scientists in her morning session and presented her findings on “Transplantation of the cerebral cortex of the albino rat”. Her research revealed that the age of the donor tissue was critical and that neonatal tissue could survive in an adult brain. Her hand drawings of the host rat brain showing the presence of new neurons and neurites were both artistic and meaningful, and undoubtedly led to thousands of subsequent publications that utilized developing neural tissue to explore the potential for repair of neurological diseases and disorders. While so many others have made profound discoveries well beyond the scope of these early works, that Dr. Hopkins-Dunn was able to move our field forward amidst professional and likely personal obstacles

suggests a brilliant mind and a persistent personality of this “First Lady of Neural Transplantation”.

*Acknowledgements:* Special appreciation to NIH since 1974, ASNTR since 1994 and Grinnell College, Northwestern University, New York University, and the University of Chicago for their historical insights on our early pioneers.

### **Rab1A and Rab3B gene therapy in the Q175 knock in model of Huntington's disease**

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Regulation of synaptic function, vesicular transport and organelle dynamics is fundamental to the normal function of the neuron. These processes are dysregulated in many neurodegenerative diseases prior to cell death, therefore providing a target for therapeutic intervention at early stages. Synaptic dysfunction and disturbances in protein transport and energy homeostasis have been reported in Huntington's disease (HD) patients, and in experimental models with a CAG expansion in the huntingtin gene. These changes are modulated by specific direct and indirect protein interactions with huntingtin and the cytoskeleton, synapses and organelles, leading to both gain and loss of function. We have previously shown that delivery of Rab3b by gene therapy can improve neurotransmitter handling and storage capacity at presynaptic terminals, and prevent synaptic neurodegenerative changes in the dopaminergic system. We hypothesize that modulation of early pathophysiological changes by Rab proteins can enhance neuronal function in experimental models of HD. We first examined motor and cognitive functional deficits, biochemical changes, inflammation, inclusion formation, and the dysregulation of multiple proteins involved in synaptic and axonal transport, in a novel Q175 knock in (Q175 KI) mouse model of HD at 1, 6, 12 and 16 months of age. Q175 KI mice exhibited motor and cognitive deficits at 12 months, which were paralleled to striatal atrophy and microglial activation. Intracellular inclusions of mutant huntingtin were present from 6 months of age. Striatal GABA levels were increased at 1 and 6 months and a reduction in striatal dopamine observed from 6-16 months. Levels of proteins involved in synaptic and cellular transport functions were differentially altered in the striatum, motor cortex, prefrontal cortex and hippocampus at 12 months, including a reduction of the synaptic vesicle protein Rab3B, and of the ER to golgi transport protein Rab1A, in cortical regions. We are currently testing whether intra-cortical and intra-striatal gene therapy of AAV2/5 Rab1A and Rab3B in Q175 KI mice will improve secretory transport and synaptic functions of the corticostriatal pathway, thereby preventing key pre-degenerative changes associated with HD.

*Funding:* CHDI Foundation.

**Midkine (MDK): a newly-recognized endogenously-produced cytokine that, through an autocrine mechanism, is pivotal for neural induction**

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Having devised a novel strategy for deriving pure neuroectoderm from human embryonic stem cells (hESCs), we performed the largest differential (phospho)proteomic analysis on any biological system to date, comparing hESCs with their neural stem cell (hNSC) derivatives to determine the key molecules responsible for neuralization & the transition from pluripotency to multipotency. Unexpectedly, MDK was exceptionally abundant where pluripotency marker OCT4 was receding, its expression highest in hNSCs. Though exogenous MDK promoted “neural tube” formation, *endogenous* MDK constitutively produced by the colony itself & acting upon receptors on OCT4<sup>+</sup> cells in an autocrine fashion was sufficient to yield pure PAX6<sup>+</sup> multipotent hNSCs (which could mature into electrophysiologically-active anterior dorsal cortical interneurons, which became caudalized with time, as well as other more specialized neural lineages, including glia, and motor and dopaminergic neurons). Inactivation of endogenous MDK (via neutralizing antibodies or shRNA-mediated knock-down) blocked neuralization. As suggested by the dataset and experimentally validated, MDK’s neural induction was mediated in part by PI3K/AKT/mTOR signaling, the inhibition of which disrupted neuralization and shifted hESC fate towards endoderm. Taken together, the (phospho)proteomic dataset and the confirmatory experiments above provide insights into a heretofore unrecognized mechanism critical to the emergence of neuroectoderm.

**TRACK-HD and TRACK-ON HD – yielding new insights into Huntington’s disease**

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Huntington’s disease (HD) is a devastating autosomal dominantly inherited neurodegenerative disease for which there is currently no effective disease modifying therapy. The genetic predictability of HD provides an opportunity for early therapeutic intervention many years before overt symptom onset and at a time when reversal or prevention of neural dysfunction may still be possible. As HD is monogenetic, fully penetrant, and characterised by a long premanifest phase, it is emerging as a potential model for studying therapeutic intervention in other neurodegenerative conditions such as Alzheimer’s or Parkinson’s disease where no preclinical diagnostic tests exist. Understanding of HD pathogenesis is evolving, and there are a number of candidate therapeutics with potential disease-modifying effects that are currently being tested. The most promising approaches will be briefly reviewed.

Since 2008 TRACK-HD has chronicled the earliest stages of the neurodegenerative disease processes in premanifest and mild to moderately symptomatic individuals who carry the HD expansion mutation (Tabrizi *et al.*, *Lancet Neurology* 2009, 2011). TRACK-HD was designed to observe natural disease progression in premanifest and early stage HD with the aim of understanding the preclinical and early phases of neurodegeneration, phenotypic correlates of neuronal dysfunction and to establish sensitive and specific clinical and biological markers of disease progression. In 2012, we reported longitudinal effect sizes for disease-progression in early stage HD over 24 months (Tabrizi *et al.*, *Lancet Neurology* 2012). Both our 24 month and recently published 36 month time-point data will be presented (Tabrizi *et al.*, *Lancet Neurology* 2013), including new insights into predictors of HD progression in both premanifest and early stage subjects, and a range of novel clinical measures that now show significant change in the premanifest group over this period. We have also identified baseline predictors of disease progression which may help enrichment for future disease-modifying clinical trials. We are now in a position to model progression in a range of functional, biochemical and imaging measures across the spectrum of disease. Finally I will summarise our ongoing research aiming to identify neural compensatory networks that may occur in the premanifest phase of neurodegeneration in HD, and dissection of the role of the innate immune system as a key modifier of disease progression.

**Growth factor gene therapy for Alzheimer’s disease: NGF and BDNF**

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Nervous system growth factors have extensive effects on neuronal function and survival. Nerve growth factor (NGF) prevents the death and stimulates the function of basal forebrain cholinergic neurons in correlational models of Alzheimer’s disease (AD), leading to its translation to Phase 1 and 2 human clinical trials. Separately, Brain-Derived Neurotrophic Factor (BDNF) influences the survival and function of entorhinal cortical and hippocampal neurons in several animal models of AD, including transgenic mutant APP-expressing mice; aged rats and lesioned rats; and aged and lesioned primates. Thus, BDNF therapy has the potential to specifically target memory deficits in AD. The beneficial effects of growth factors in AD models appear to occur independently of detectable alterations in beta amyloid load, providing therapeutic alternatives to A $\beta$ -modifying therapies in AD. This lecture will provide an update on the current status and future directions of neurotrophic factor therapies that are in preclinical and clinical development.

**Adult stem cell-based therapy for ischemic stroke**

Catherine M. Verfaillie  
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Neural stem cells or neural stem/progenitor cells differentiated from pluripotent stem cells can generate neurons, astrocytes and oligodendrocytes, which could be used for regeneration of lost neural tissue following stroke, even if this has yet to be accomplished. Other cell populations are also being considered for therapy of stroke. We will describe the potential of (stem) cells from non-neural postnatal tissues, such as bone marrow, which themselves do not (or maybe in a very limited manner) generate neural progeny. However, these non-neuroectoderm derived cell populations may induce endogenous neurogenesis and angiogenesis, as well as have immunomodulatory properties that improve stroke outcomes. Preclinical animal studies (and pitfalls), early clinical studies, and possible mechanisms of action, as well as possible unforeseen complications will be discussed.

**From pluripotent stem cells to cortical circuits: perspectives for disease modelling and brain repair**

Pierre Vanderhaeghen

*Bruxelles, Belgium*

The cerebral cortex consists of several hundreds of different types of neurons, organized into specific cortical layers and areas, that display specific profiles of gene expression, morphology, excitability and connectivity.

Embryonic stem (ES) and other pluripotent stem cells constitute a promising tool for the modelling and treatment of human neural diseases.

Here we describe an intrinsic pathway by which pluripotent stem cells, whether of mouse or human origin, recapitulate *in vitro* the major milestones of cortical development, leading to the sequential generation of a diverse repertoire of pyramidal neurons that display most salient features of genuine cortical neurons.

Interestingly, the mouse and human pathways of corticogenesis display many similarities but also striking differences that may be related to species-specific developmental programmes.

When transplanted into the cerebral cortex of new-born mice, the ESC-derived cortical neurons develop specific patterns of axonal and dendritic projections corresponding to endogenous cortical projections *in vivo*. Following transplantation into lesioned adult mouse cortex, the grafted neurons also establish robust and specific long range projections and synapses corresponding to cortical circuits.

These data shed new light on the mechanisms of neuronal specification, and constitutes an innovative tool to model human cortical development, evolution, and disease, *in vitro* and *in vivo*. In the long run, cortical neurons generated *in vitro* could be used also in the perspective of brain repair, for several diseases striking cortical neurons.

**Production of new striatal neurons in the healthy and diseased neonatal brain**

J. Wright and L.H. Thompson

*Melbourne, Australia*

Substantial advances have been made in the last decade on our understating of the basic physiology underlying neurogenesis in the postnatal mammalian brain. The bulk of the work in this area has been based on analysis of the adult brain. Relatively less is known about the capacity for neurogenesis in specific structures within the neonatal brain. Here we demonstrate that the production of medium spiny striatal projection neurons (MSNs) in the striatum extends into the early neonatal period under normal physiological conditions in the rat brain, with low but stable contributions of striatal interneurons during this time-period. Using retroviral labeling, we further show how MSNs born neonatally, correctly acquire their axonal targets in the globus pallidus and midbrain. These data provide evidence of latent MSN development in the neonatal striatum post-embryogenesis and as such, these new born cells provide promising targets for gene therapy to promote endogenous self-repair during neonatal striatal injury such as in cerebral palsy. Based on this, we are developing a novel hypoxia-independent neonatal stroke model in order to compare our results with striatal neurogenesis in a damaged brain which will give further insight into whether MSN production is enhanced or inhibited due to striatal damage.

**ABSTRACTS OF POSTER PRESENTATIONS**

**Generating patient-derived Parkinsonian iPS cell lines, their differentiation to midbrain dopaminergic neurons and susceptibility to neurotoxic insults**

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Parkinson's disease (PD) is neurodegenerative disease clinically characterised by the loss of A9 dopaminergic neurons in the substantia nigra pars compacta (SNc) of the midbrain. Using patient-derived stem cells we aim to model the disease by recapitulating the neurons lost in PD and investigate their susceptibility to neurotoxic insult. Dermal fibroblasts from idiopathic and genetic PD patients were transduced with lentiviruses to generate induced pluripotent stem (iPS) cell lines. We have developed a robust protocol for the generation of dopaminergic neurons of the SNc and have characterised these neurons from lines generated for the markers of SNc and their dopamine release. We have then subjected these neurons to neurotoxic insults to model PD *in-vitro* and are currently looking at ways to protect these *de-novo* neurons from toxicity and prevent their degeneration.

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## Understanding rejection of allogeneic and xenogeneic dopaminergic cell transplants

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A restorative approach for Parkinson's disease (PD) is the transplantation of foetal ventral mesencephalon (VM). Post-mortem studies of patients treated in previous clinical trials have revealed the presence of infiltrated immune cells [1], which may result from a lack of immune compatibility between the donor cells and the host. The impact this may have on the functional outcome of cell therapy for PD has yet to be determined. To address this question, it is necessary to establish a controlled model of rejection so this work compared the rejection of allograft and xenograft transplants in rat hosts. 6-OHDA unilaterally lesioned Sprague Dawley received intrastriatal VM transplant derived from embryos of CD1 mice (xenograft) or Wistar rats (allograft). To establish the time course of rejection, xenografted animals received immunosuppressive treatment (cycloA) for different durations, while rejection of the allograft was obtained by peripheral injections of spleen cells [2]. Rotational behavior in response to amphetamine was used as a behavioural assessment of graft survival alongside immunohistochemical analysis. The immune response, determined by infiltration of leukocytes, appears within the second week of withdrawal of immunosuppression, although no significant cell loss was observed before the 3rd week. Regarding the allograft model, the use of different time points for spleen cells injections induced different degrees of rejection, from 23% to 96% grafted cell loss, 4 weeks post-graft, therefore creating a more controllable model than the xenograft model.

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## A potential compensatory role for endogenous striatal tyrosine hydroxylase-positive neurons in a non-human primate model of Parkinson's disease

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The neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) selectively destroys dopaminergic (DA) neurons in the nigrostriatal pathway while importantly sparing other DA systems, serving as a significant tool

in Parkinson's disease (PD) research. Extensive studies in our laboratories have shown reversal of MPTP induced PD symptoms following striatal grafts of fetal ventral mesencephalic DA neurons in the African Green monkey. Additionally, our and other studies have reported an upregulation of endogenous tyrosine hydroxylase (TH) positive neurons in the striatum following MPTP lesions (~140% increase). The aim of the current research is to investigate the fate of this endogenous population in MPTP treated monkeys following fetal cell grafts. Preliminary results indicate a return to control levels for the TH-positive cells following successful transplantation, while animals whose donor cell grafts failed to contain DA neurons retained the elevated levels. If these cells represent a compensatory mechanism in an attempt to replenish DA in the striatum, then the ability to influence this population could prove beneficial in developing new non-invasive therapeutic treatments. Current research investigating the origin of these cells and their relationship to symptom severity is ongoing. Supported by an Academic Enrichment Grant from the University of Colorado School of Medicine, The Axion Research Foundation, and 5PO1NS044281.

## Human Induced Pluripotent Stem (iPS) Cells for Cell Replacement Therapy in Huntington's disease

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Huntington's disease (HD) is a neurodegenerative disease caused by a mutation in the huntingtin gene (HTT). The extended CAG repeat ultimately leads to loss of medium spiny neurons (MSNs) in the striatum of the HD brain. Cell replacement therapy using primary human fetal tissue has shown 'proof of principle' as a strategy to treat this genetically inherited disease [1]. However, alternative cell sources need to be identified to overcome the ethical and logistical issues that are associated with using human fetuses. IPS cells were first introduced by Yamanaka in 2006 by direct reprogramming of fibroblasts to ES-like cells by using four defined factors (Oct4, Sox2, Klf4, c-Myc) [2]. Here we attempt to generate safety iPS cells by introduce reprogramming factors using *piggyBac* Transposon [3] transduction system to human fetal fibroblasts and fetal neural stem cells which theoretically have the potential to be more readily reprogrammed and are genetically unrelated to the host. The establishing iPS cell line were present the similarities to human embryonic stem (ES) cells in morphology, surface antigen, and proliferation.

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### Differential neuroprotective capacity of Endothelial Progenitor Cells-derived factors

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Stem and progenitor cells release a wide array of trophic factors, cytokines and extracellular matrix molecules. The present study aimed at investigating whether endothelial progenitor cells (EPC) may support neuronal functions and survival by means of paracrine factors.

EPC isolated from peripheral blood of healthy human donors were cultured in hypoxic conditions to stimulate secretion of growth factors. Primary cultures from fetal rat embryonic (E14) ganglionic eminence (GE) and ventral mesencephalon (VM) were treated with EPC derived conditioned medium (EPC-CM). Incubation of cultures with EPC-CM resulted in a significant increase in TH-ir cell densities in VM as well as GABA-ir cell densities in the GE cultures, respectively. Interestingly, treatment of cultures with EPC-CM resulted in a substantial increase in number of microglial cells. Notably, EPC-CM displayed neuroprotection against MPP<sup>+</sup> toxicity in VM cultures whereas it was not effective against 3NP toxicity. The effect of EPC-CM on TH-ir cells persisted upon treatment with Ara-C conducted in order to limit microglia expansion.

Our findings identified EPC-CM as a powerful tool to promote viability and/or differentiation of neuronal cells. EPC-CM constituents might represent a new strategy to support neuronal repair.

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### Characterisation of FoxP1 in the striatum

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Medium Spiny Neurons (MSNs) constitute >90% of the projection neurons in the striatum and are lost in Huntington's disease (HD). A screen looking at gene expression changes in the developing striatum of mice (E12-E16) showed *FoxP1* to be the most up-regulated gene over this period. *FoxP1* is also expressed in the adult striatum and has the potential to be a marker of both developing and mature MSNs (Tamura *et al.*, 2003). Understanding the regulation of striatal development, in particular, the differentiation of MSNs is the key to finding strategies to 'direct' the differentiation of such cells from pluripotent stem cells. This in turn is crucial for generating genuine striatal cells for cell therapy in conditions such as HD.

To date the function of *Foxp1* in the brain is unknown. Homozygous FOXP1 knock-outs (*FoxP1*<sup>-/-</sup>) in mice are embryonically lethal from ~E15 due to cardiac defects (Wang *et al.*, 2004). However, one can analyse the embryos before the onset of lethality. Using several techniques we have attempted to understand what role *FoxP1* has in MSN

differentiation. Results show that there is a decrease in the number of DARPP-32 expressing neurons when FoxP1 is absent. In addition, ongoing microarray analysis will highlight differences at a genomic level, between the *FoxP1*<sup>-/-</sup> nulls and WTs in the striatum at E14.

### Birth dating of dopamine neuron subtypes in ventral mesencephalon grafts in a rat model of Parkinson's disease

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Using co-expression of tyrosine hydroxylase (TH) with GIRK2 and Calbindin1 to identify A9 and A10 dopamine neuron subtypes, respectively, we have previously shown that grafts derived from younger ventral midbrain donor tissue are enriched with A9 neurons. It is unclear however, whether this is due to different survival levels or differentiation of dopamine neuron precursors after transplantation.

Here we used BrDU labelling to birth date rat embryonic day 12 (E12) and E14 dopamine neurons after transplantation into the striatum. The results show that younger donor grafts contained significantly more mitotic dopamine neuron precursors, which continued to proliferate in the host striatum and two thirds of which were able to mature into A9 neurons, a third differentiated into A10 neurons. These findings demonstrate that a higher yield of functionally important A9 neurons in younger donor grafts is, at least in part, due to continued dopamine cell division after transplantation. This has significant implications for the generation and selection of dopamine precursors at the right developmental stage from stem cell based sources to improve cell transplantation therapy in PD.

*Acknowledgements:* This work was funded by the Wellcome Trust.

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### Vitamin D3 promotes dopamine neuron survival through upregulation of GDNF

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Vitamins play a key role in brain development and neuronal survival. In previous work using an iTRAQ proteomics technique and immunohistochemistry we identified vitamin D3 receptor expression during peak neurogenesis of dopamine neurons in the ventral midbrain.

In this study we applied the active metabolite of vitamin D3, calcitriol, to cultures of embryonic day 12 ventral

mesencephalon neurons to investigate its role in neuronal differentiation and survival. Calcitriol enhanced the total number of tyrosine hydroxylase-positive dopamine neurons in a dose dependent manner, up-regulating GDNF, and proffering neuroprotection rather than generating new dopamine neurons. Blocking GDNF signalling reversed calcitriol's positive effect on enhancing the number of dopamine neurons.

We then treated E12 ventral mesencephalic cell suspensions with vitamin D3 - either as calcitriol to the transplant suspension or vitamin D3 delivered orally - prior to transplantation in 6-OHDA lesioned rats. Although all grafts reversed amphetamine-induced rotation to a similar degree, vitamin D3-treated grafts gave earlier recovery in the cylinder test compared with control transplants. Transplant histology to assess vitamin D3's effects on dopamine neuron survival and graft-host innervation will be presented.

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#### **The influence of nicotinamide on directed differentiation of neurons from embryonic stem cells in vitro**

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Factors controlling proliferation and differentiation are crucial towards advancement of neural cell-based experimental neurodegenerative therapies. In this regard, nicotinamide has been shown to function in neural cell fate determination, enhance neuralization and influence DNA repair and apoptosis.

This study investigated whether nicotinamide could direct the differentiation of mouse embryonic stem cells (mESCs), cultured as monolayers, into neurons. 5 mM and 10 mM nicotinamide added at days 0-7 and 7-14 significantly increased  $\beta$ -III tubulin positive neuronal populations, concomitantly decreasing the total number of cells in culture measured by quantification of 4',6-diamidino-2-phenylindole (DAPI) positive cells. Interestingly, nicotinamide had a more significant effect on neuronal differentiation at day 0-7. Current work is focusing on elucidating the mechanism mediating neural specification by nicotinamide, i.e. induction of cell-cycle exit and/or selective apoptosis in non-neural populations. Preliminary data indicates a reduction in the proportion of proliferating cells in nicotinamide-treated cultures, suggesting that nicotinamide may enhance cell-cycle exit thereby promoting neuronal differentiation. We are currently investigating the effect of nicotinamide on the process of neural induction and whether it influences neuronal subtypes generated *in vitro*.

Future work will focus on evaluating the effect of nicotinamide on the differentiation of midbrain dopamine neurons; towards a therapy for Parkinson's disease.

#### **Brain endogenous Liver X Receptor ligands selectively promote midbrain neurogenesis**

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Liver X receptors (Lxr $\alpha$  and Lxr $\beta$ ) are ligand-dependent nuclear receptors critical for ventral midbrain neurogenesis *in vivo*. However, no endogenous midbrain Lxr ligand has so far been identified. Here we employed LC-MS and functional assays to identify cholic acid as a novel Lxr ligand. Moreover, 24(S),25-epoxycholesterol (24,25-EC) was found to be the most potent and abundant Lxr ligand in the developing mouse midbrain. Both Lxr ligands promoted neural development, in an Lxr-dependent manner, in zebrafish *in vivo*. Intriguingly, each ligand selectively regulated the development of distinct midbrain neuronal populations. While cholic acid increased survival and neurogenesis of Brn3a + Red Nucleus neurons, 24,25-EC promoted dopaminergic neurogenesis. These results identify an entirely new class of highly selective and cell-type specific regulators of neurogenesis and neuronal survival. Moreover, 24,25-EC promoted dopaminergic differentiation of ES cells, suggesting that Lxr ligands may thus contribute to the development of cell replacement/regenerative therapies for Parkinson's disease [1].

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#### **Safety study on cyclosporine A in epilepsy models**

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Neural transplantation of inhibitory cells into seizure initiating or propagating brain regions is one promising approach to treat pharmacoresistant epilepsies. Depending on the grafted cell type (e.g. xenotransplantation), immunosuppression is necessary to prevent graft rejection, thereby enabling long-lasting anticonvulsant effects of the graft.

We therefore investigated the effects of daily treatment (15 days) with different preparations of the commonly used immunosuppressive drug cyclosporine A (CsA) (pure substance or ready-to-use-drug *Sandimmun*<sup>®</sup>, Novartis), doses (5 or 10 mg/kg), and application routes (i.p. vs. s.c.) on acute and chronic seizure thresholds in rats. Additionally, CsA whole blood levels were analyzed and behavioral tests were conducted to detect side effects.

The data did not reveal acute and only subtle chronic effects of immunosuppression with either pure CsA or *Sandimmun*<sup>®</sup>



on seizure thresholds. The resorption of i.p. applied CsA from *Sandimmun*<sup>®</sup> exceeded the resorption from the pure CsA preparation. Unwanted side effects included transient gastrointestinal problems. Our data indicate that immunosuppression with 10 mg/kg *Sandimmun*<sup>®</sup> i.p. rather than pure CsA is a safe and feasible option for use in neural transplantation experiments in epilepsy models.

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### **Are transplant-induced improvements in cognitive performance in the rat lesion model of Huntington's Disease dependent on frontal-striatal circuit reconnection?**

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Frontal-type executive function, demonstrated in rats using a delayed alternation (DA) task, is disrupted when continuity of both cortico-striatal loops of the associated neural circuitry are interrupted. Bilateral grafts of embryonic whole ganglionic eminences (WGEs) restore function in the DA task in bilateral striatally lesioned rats, with transplanted tissue integrating with the host brain. However, it has not been definitively shown that improvement in cognitive performance is due to physical reconstruction of neural circuitry or if grafted tissue simply exhibits other beneficial influences on the host brain, for example by releasing donor derived growth factors. Therefore, in the present study, we aim to determine the role of circuit reconstruction in the attenuation of cognitive deficits. This is to be assessed by training rats on the operant DA task and evaluating performance following bilateral striatal lesions, unilateral striatal transplantation of WGE, and ipsilateral or contralateral surgical disconnection of the prefrontal cortico-striatal circuitry.

We hypothesise that unilateral grafts should provide effective alleviation of the bilateral striatal lesion deficit, and that contralateral knife cuts will preserve prefrontal cortical afferents to the grafted striatum thus maintaining cognitive performance, whereas ipsilateral disconnection of the graft will reinstate the deficit. The data presented represents the current status of the study.

This work is supported by the MRC.

### **Optogenetic manipulation of hESCs and *in vivo* measurement of dopamine release**

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Martin Lundblad and Malin Parmar  
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Human embryonic stem cells (hESCs) are a possible alternative to primary fetal tissue for the transplantation of dopamine neurons into patients with Parkinson's disease

(PD). We aim to investigate the contribution of engrafted hESC-derived mesencephalic dopamine (mesDA) neurons on functional improvements in a rat model of PD.

In this study we use Channelrhodopsin (ChR2) allowing us to manipulate cell activity and release DA with light pulse stimulation, at a high temporal resolution. By transducing hESCs with an LV-vector to express ChR2 under the synapsin-1 promoter (Syn), transplanted cells will express the construct in neurons.

Here we engrafted one group of 6-hydroxydopamine lesioned rats with H9-hESC-Syn-ChR2 cells that were patterned towards a mesDA phenotype whilst having a lesioned group serving as control. We will assess cell survival and dopamine release of transplanted cells via amphetamine-induced rotations as well as using *in vivo* amperometry in conjunction with optogenetic stimulation, in order to manipulate neural activity. At the conference we will present the first findings of this study. It is hoped the results from this study will provide insight into the neurochemical events that underpin graft-mediated DA-release/functional recovery in rat models of PD.

### **Influence of transcranial direct current stimulation on the survival, migration and integration of dopaminergic cell transplants in a rat model of Parkinson's disease: a histological and behavioral analysis**

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*Introduction:* Parkinson's disease (PD) is a challenging disease, which leads to progressive and disabling deterioration of motor and cognitive skills. Transcranial direct current stimulation (tDCS) is a non-invasive technique that presents modulatory effects on cognitive functions and motor behavior. Regenerative approaches are under research to reconstitute dopaminergic neurotransmission and offer a more extensive and long lasting effect. In this study we analyzed the effect of tDCS on the survival, migration and integration of dopaminergic cell transplants.

*Methods:* Rats received unilateral dopamine-denervating lesions using 6-hydroxydopamine. Afterwards, they were randomly assigned into three groups (Anodal-Stim, Cathodal-Stim and Sham-Stim) according to values in amphetamine-induced rotation. All groups were transplanted with E14 GFP<sup>+</sup> ventral mesencephalic (VM) cells into the ipsilateral striatum. tDCS was started right after transplantation and performed for 20 min daily, during 2 weeks. Amphetamine-induced rotation and cylinder test were performed pre transplantation and 5 weeks after transplantation, respectively. Rats were perfused 5 weeks after transplantation and histological analysis was performed.

*Results:* Survival of transplanted VM cells in the striatum was increased by approximately 50% in the anodally

stimulated group as compared to sham and cathodal groups, and optical fiber density was increased in the anodal tDCS-group. All groups improved their scores in amphetamine-induced rotation, but there were not improvements in the cylinder test at this early time-point after grafting.

**Conclusion:** Transcranial direct current stimulation improved graft survival and fiber outgrowth of intrastriatal dopamine grafts in a rat PD model. Further studies are ongoing to confirm and extend these findings.

### **Trefoil Factor 1 in the nigrostriatal system of 6-hydroxydopamine-lesioned rats**

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Trefoil factor 1 (TFF1) belongs to a family of secreted peptides mainly expressed in the gastrointestinal tract. TFF1 has also been suggested as a neuropeptide, but not much is known about its expression and function in the CNS.

We investigated the expression of TFF1 in control and 6-hydroxydopamine (6-OHDA)-lesioned rats. In the unlesioned ventral mesencephalon, TFF1-immunoreactive (-ir) cells were mainly found in substantia nigra pars compacta (SNc) and in the ventral tegmental area. While around 90% of the TFF1-ir cells in SNc co-expressed tyrosine hydroxylase (TH), only a subpopulation of TH-ir neurons expressed TFF1. Some TFF1-ir cells in SNc co-expressed calbindin or calretinin, and nearly all were NeuN-ir while there was no co-localization with the astroglial marker GFAP. A few TFF1-ir cells were also found in the striatum, and their numbers significantly increased after 6-OHDA lesion.

Intrastriatal injection of Fluorogold resulted in retrograde labeling of several TFF1-ir cells in the SNc showing that these cells were projection neurons. This was also reflected by unilateral loss of TFF1-ir cells in the SNc of 6-OHDA-lesioned rats.

Our findings demonstrate that distinct subpopulations of midbrain dopaminergic neurons express TFF1 and that this expression pattern is altered in a rat model of Parkinson's disease.

### **Comparison of L-Dopa response patterns of different stages of MSA-P in a double lesion rat model**

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The objective of this study was to identify the striatal sub-region and QA dosage replicating L-Dopa failure in a

partial double lesion rat model of MSA-P. Three experimental groups were investigated: (1) PD group receiving 6-OHDA lesion, (2) MSA-P mild (3) MSA-P severe group receiving 6-OHDA and QA lesion. Following 6-OHDA lesion all groups showed significant behavioral deficits in cylinder and stepping test, without further deterioration after QA lesion except MSA-severe group with significant impairment in stepping test. Amphetamine induced rotation resulted in ipsilateral rotation of PD and MSA groups, whereas apomorphine induced contralateral rotation in PD and ipsilateral rotation in MSA groups. All groups revealed a significant L-Dopa response in cylinder and stepping test after 6-OHDA lesion that was significantly reduced in both MSA groups following the subsequent QA lesion, with a trend of MSA-P severe group being most resistant to L-Dopa. In conclusion, the current study describes the unique motor behavior and L-Dopa response patterns in association to the location and the dosage of the QA lesion of double lesioned animals compared to single 6-OHDA lesioned animals. Both QA lesion approaches in MSA-mild and MSA-severe groups led to a reduction of L-Dopa responsiveness, whereas the higher dose of QA showed the greatest reduction of such. This refined model of MSA-P replicating L-Dopa failure is highly suitable for future grafting studies with the rationale of restoring L-Dopa responsiveness.

### **Cell intrinsic and extrinsic factors contribute to enhance neural circuit reconstruction following transplantation in Parkinsonian mice**

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Homotopic grafting into the SNpc is capable of restoring the nigrostriatal pathway and forming appropriate connections with the striatum, but the extent of striatal reinnervation remains substantially less than can be achieved with ectopic placement. The aim of this investigation was to determine what effect donor age and virally delivered glial derived neurotrophic factor (GDNF) over-expression had on the survival, growth and integration of homotopic grafts placed into 6OHDA mouse model of Parkinson's disease. Younger donor tissue (E10) showed to be capable of generating larger grafts with more axonal growth compared to grafts generated from the more conventional E12 donor VM. With the addition of GDNF, fiber growth and striatal innervation was further enhanced in both older and younger donor tissue. Immunohistochemical staining revealed that younger donor grafts treated with GDNF resulted in an increased number and proportion of A9 DA neurons. Behavioural testing confirmed that younger donor grafts provided a significant improvement in motor behavior with GDNF overexpression only affecting E12 donor tissue. These findings could have significant implications

for the future development of cell replacement therapy for the treatment of Parkinson's disease.

**Neonatal immune-tolerance in mice does not prevent xenograft rejection: a comprehensive analysis using in vivo luciferase tracking**

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While *in vitro* iPSC models are proving extremely informative, an *in vivo* "humanized" chimeric animal model of disease via transplantation of diseased human iPSC-derived cells may prove ideal for therapeutic screening and mechanistic discovery. One of the major challenges for the field is appropriate immune suppression in these xenograft models as it is often ineffective or cost-prohibitive for long term studies, and has also been shown to ameliorate neurological diseases (Rosenstock *et al.* Neurochem Int. 2011), complicating experimental results. With the promise of transplantation for neurodegenerative diseases, there also is the need for non-invasive *in vivo* tracking.

In the current collaborative study we used a range of techniques and cells to establish tolerance of the neonatal and adult rodent brain to neural xenografts. We show that in contrast to rats, mouse neonates are sensitive to human neural xenografts (from iPSCs, ESCs or fetal tissues). In three mouse strains, prior sensitization had no effect on the severe rejection of the cells. Luciferase imaging was shown to be a powerful predictor of graft survival in the striatum. Together these studies show that neonatal mice reject human cells, and that immune tolerance techniques are not sufficient to prevent rejection in adult mice.

**Human fetal dopaminergic precursor cell transplantation: LIF-nanotherapy to promote graft survival.**

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LIF (Leukaemia Inhibitory Factor) is both a neuropoietic and a tolerogenic cytokine. Thus LIF represents a promising candidate for treating neural cell grafts to promote their survival whilst simultaneously suppressing any immune reactivity against the graft.

Since *in vivo* use of LIF is hindered by its rapid degradation and excretion, we have prepared a nano-particulate formulation of LIF intermixed with PGLA, a biocompatible, biodegradable polymer similar to that used in soluble

sutures. These "LIF-nano" are decorated with avidin allowing attachment of biotinylated anti-Thy1 to bind the particles to neural precursor cells prior to grafting.

LIF also plays a role in supporting neural stem and precursor cells, and may provide dual therapeutic benefits for cell transplantation in Parkinson's Disease (PD). The TransEuro Project (<http://www.transeuro.org.uk>) is currently evaluating grafts of human fetal ventral mesencephalon (hfVM, rich in precursor dopaminergic cells) in treatment of PD. Working in parallel, we have shown that, *ex vivo*, LIF-nano-treated hfVM yield over 3-fold more dopaminergic cells compared to empty-nano controls. Similarly, *in vivo*, intra-striatal hfVM grafts in nude rats show major beneficial effects of LIF-nano therapy, both in graft survival and in dopaminergic cell maturation.

This has major implications for clinical transplantation, reducing numbers of cells required for therapeutic effect.

This work is supported by an NIHR i4i Grant.

**Simultaneous in vivo monitoring of transplanted cells and biomaterials by magnetic resonance imaging**

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Non-invasive *in vivo* monitoring of regenerative medicine approaches for tissue and organ reconstruction will be an important technological development to ensure the safety and efficacy of such strategies. However, the involvement of multiple types of cells as well as biomaterials complicates *in vivo* monitoring that is dependent on a single contrast mechanism. Herein we explored the use of chemical exchange saturation transfer (CEST) to specifically visualize different elements (multiple cell types and biomaterials) used for an *in situ* regenerative medicine approach for the treatment of the stroke-damaged brain. Specifically, neural stem cells and endothelial cells were labelled using paramagnetic CEST (PARA-CEST) agents, whereas an extracellular matrix (ECM)-derived bioscaffold was detected using diamagnetic CEST (DIA-CEST). Intracellular incorporation of PARA-CEST agents (Eu-HPDO3A and Yb-HPDO3A) was titrated to exert minimal biological effects on the cells while ensuring sufficient levels were achieved to afford detection *in vitro* and *in vivo*. Z spectra were acquired for labelled cells as well as ECM bioscaffold prior to *ex vivo* and *in vivo* imaging in a rat model of stroke. Although significant challenges remain, these results provide proof-of-principle that non-invasive imaging of tissue engineering in the brain is feasible.

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### **EGFP-Flag transfected rat progenitor cells display electrophysiological properties of integrated functional dopaminergic (DA) neurons similar to genetically labelled DA-neurons *in vitro* and intrastrially xenografted DA-neurons in a rat model of Parkinson's disease**

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Whole cell patch clamp studies of intrastrially grafted EGFP-Flag transfected ventral mesencephalic (VM) progenitor cells in brain slices displayed 2 types of neurons showing dopaminergic (Da)-like properties marked by the hyperpolarisation activated anomalous rectification. Type-1 neurons display hyperpolarisation activated rectification followed by delayed, single action potentials, and pacemaker activity. Type-2 neurons attract attention by distinct spontaneous bursting and bursting after hyperpolarisation. To clarify whether one or both Da-like neurons are actually dopaminergic, fetal VM progenitor cells of a transgenic TH-GFP mouse were used to visualise Da neurons, after grafting in a 6-OHDA-rat model, directly due to expression of fluorescent labelled tyrosine hydroxylase. These TH-GFP mice-derived Da-neurons we previously electrophysiologically characterized *in vitro*.

Since 6-OHDA lesion induces reduction in the ability to use the contralateral paw, an evaluation of motor skills by a staircase test can monitor functional integration of the grafts. This is an alternative behavioural test to drug-induced rotation, which might influence the electrophysiological properties *in situ*.

Overall, patch clamp analysis of genetically labelled Da-neurons together with behavioural studies facilitate the direct investigation of functional properties of Da neurons *in vitro* and after intrastriatal grafting.

### **Histopathological effects of varying the impact trajectories in experimental model of TBI**

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About 1.7 million people sustain TBI annually leading to significant disability and death around the world. Most of the TBI animal models focus on a single 90-degree trajectory affecting a specific brain area; but TBI patients do not always display injury to the same brain regions. We examined histopathological effects at 3 days after controlled cortical impact (CCI) model in adult Sprague-Dawley rats. We hypothesized that different impact

trajectories would produce varying levels of brain damage. CCI manipulations included: 1. conventional TBI targeting frontal cortex, 2. farthest right angle (FRA) and 3. closest right angle (CRA) both also targeting frontal cortex, 4. olfactory bulb (OB), and 5. cerebellar (CB) injury. Data showed typical cell loss in M1 cortical region in the conventional TBI, FRA, and CRA groups using H&E staining and Cavalieri method. No significant cell death was detected in M1 region of animals that received OB or CB injury. Comparable CA3 hippocampal cell loss in both hemispheres was noted across TBI groups. Increased expression of activated microglial marker OX-6 in M1 region accompanied conventional TBI, FRA, and CRA suggesting a critical inflammatory role. These results provide a closer clinical approximation of varying TBI histopathological outcomes following different impact trajectories.

*Acknowledgements:* United States Department of Defense.

### **Characterizing GDNF regulation and its impact in the 6-OHDA rodent model**

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Destabilizing domains (DD) regulate gene expression by targeting specific proteins fused to DD for destruction. More importantly, DD-regulated transgene expression can be stabilized using small molecules such as trimethoprim.

Lentiviral vectors were used to deliver regulated glial cell-derived neurotrophic factor (GDNF-DD) to the striatum of rats. The expression of GDNF-DD was then turned ON using TMP in the drinking water. Three weeks after the expression was turned ON, the animals were lesioned with 6-OHDA.

Initial studies showed that GDNF-DD was able to provide neuroprotection of substantia nigra neurons to levels comparable to wild-type GDNF. Histological analysis for GDNF activity using phosphorylated ribosomal protein S6 (pS6) showed a significant increase in the number of surviving pS6 positive nigral cells of 140% in the GDNF group and 77% in the GDNF-DD group where expression was turned ON. When the expression of GDNF-DD was turned OFF, only 30% of pS6 positive cells remained in the lesioned substantia nigra. This decrease was comparable to control groups.

To determine if GDNF-DD expression could ameliorate or stop an ongoing degeneration or a fully lesioned brain, the expression of GDNF-DD was turned ON right after 6-OHDA lesion or 4 weeks after the animals were lesioned with 6-OHDA.

Amphetamine-induced rotations were performed before lesion, 4 weeks after lesion and 9 weeks after lesion. In

both groups where GDNF-DD expression was turned ON after immediately after lesion and 4 weeks after lesion there was a maintenance in the level of rotations. This was in contrast with control groups, where there was an increase of rotations through time, which reflected a progressive degeneration of SNpc.

Histological analysis using pS6 also indicated a significant increase in the number of pS6 positive cells in both groups. The results indicate that the DD system is a promising tool for regulating GDNF *in vivo*.

#### **Neonatal desensitisation to human embryonic tissue can be induced using a range of tissue types**

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Prior to clinical application; safety and efficacy of potential human donor cells for transplantation in neurodegenerative disease must first be demonstrated in animal models. Neural xenografts are rejected by the rodent immune system within three weeks of implantation. Although immunosuppressant drugs are mostly effective short-term, toxic side-effects lead to termination of experiments before human cell differentiation. Immunodeficient hosts cannot withstand rigorous functional testing, rendering current methods inadequate for full preclinical assessment. We have developed a novel method in which host animals are “desensitised” to xenogeneic donor cells via an injection during the neonatal period, promoting acceptance of human neural xenografts in adulthood.

Successful desensitisation has been demonstrated in rats using neonatal injections of human neural tissue. Desensitising with non-neural cells would release more valuable neural tissue for transplantation and would also inform on the immunological mechanisms underlying neonatal desensitisation. We compared survival of human transplants in the rat striatum in hosts desensitised neonatally with different human foetal tissues. Results suggest it may not be necessary for desensitising cell suspensions and transplanted cells to match, but that some tissue types may be more effective for desensitisation to xenogeneic tissue.

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#### **Controlled release of neurotransmitters from biopolymer matrices influence neural stem cell proliferation and distribution**

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Spinal cord injury (SCI) results in cavity formation and the loss of nervous tissue. Possible treatment can combine stem cells with matrix support and drug release in order to optimise the regeneration of lesioned tissue.

We have utilized a heterogenic hydrogel system which releases cationic promoters that influence neural growth. The heterogenic ion exchanger Dowex 50WX8 was scattered in porous hydrogels based on 2-hydroxyethyl-methacrylate or 2-hydroxypropylmethacrylamide. Neurotransmitters and/or their analogs (carbachol, serotonin, L-dopamine and tryptamine) were bound to the ion exchanger. The release of carbachol and tryptamine increased cell proliferation by 20–30% compared to neural stem cells cultured in the presence of a control gel. Dopamine and serotonin significantly decreased cell proliferation. The growth and distribution of the cells in all hydrogels were analyzed immunohistochemically after 2, 7, 14, 21 and 28 days. We demonstrated that the cells were unable to attach and grow on the gel surface in the presence of dopamine, whereas tryptamine and carbachol supported both cell attachment and growth during 28 days.

Our *in vitro* results demonstrate that the most suitable candidate for further *in vivo* experiments examining the combined treatment of SCI is a heterogenic hydrogel system releasing carbachol.

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#### **Transplantation of fetal ventral mesencephalic progenitor cells overexpressing high molecular weight FGF-2 isoforms in 6-OHDA lesioned rats**

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FGF-2 is one of the most potent neurotrophic factors to promote survival of dopaminergic (DA) neurons. It is expressed in different isoforms representing different translation products from a single mRNA. For this study we selected the high molecular weight (HMW) isoforms (21/23 kDa) since we could show that HMW FGF-2 transfected ventral mesencephalic (VM) cell preparations contained up to 3 times more DA neurons. After expansion and differentiation *in vitro*, HMW FGF-2 overexpressing embryonic day-12 VM cells were transplanted unilaterally into 6-OHDA lesioned rats.

Goal of this ongoing study is to analyze the effects of HMW FGF-2 produced by the transfected VM cells as their “own” neurotrophic factor. The experimental design compares three grafted groups (HMW FGF-2 transfected, empty control vector transfected and non-transfected cells) with a lesioned control group. In addition, two different transplantation paradigms with a different amount of grafted cells are under evaluation. Animals are analyzed for behavioural performance for up to 12 weeks post transplantation and immunohistochemical measurements of DA neuron numbers and striatal re-innervation are studied. Characterizing these grafts will aim to advance understanding of the influence of

transfected neurotrophic factors on survival and differentiation of DA cells.

### **Antagonizing Nogo-receptor 1 promotes the number of cultured dopaminergic neurons and elongates their neurites**

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The myelin associated protein Nogo-A and its receptor NgR1 are among others the most potent growth inhibitors of the adult CNS. Nogo-A is mostly expressed on the surface of oligodendrocytes, but is also found in neurons of the adult and developing CNS. This suggests that Nogo-A serves additional functions in the brain. In the present study, we investigated the effects of antagonizing NgR1 on dopaminergic neurons. For that purpose ventral mesencephalic (VM) cultures from E14 rat embryos were grown in absence or presence of the NgR1 antagonist NEP1-40 for one week. Treatment with NEP1-40 significantly increased cell density of tyrosine hydroxylase (TH)-immunoreactive neurons. Morphological analysis of TH-positive neurons disclosed longer neurites and higher numbers of primary neurites in cultures incubated with NEP1-40, while soma size was not changed. Moreover, organotypic VM cultures displayed significantly bigger volume and higher TH-positive cell numbers after NEP1-40 treatment. Similarly, Western Blot analyzes showed increased expression levels of TH after NEP1-40 administration.

In sum, our findings demonstrate that the intervention of Nogo-A signalling by antagonizing NgR1 modulates dopaminergic neuron properties in the developing mid-brain. These observations might have substantial impact in the context of Parkinson's disease. Supported by SNF and the Swiss Parkinson Foundation.

### **The anti-dyskinetic effect of dopamine receptor blockade is enhanced in parkinsonian rats following dopamine neuron transplantation**

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We have previously shown that low doses of buspirone (5HT<sub>1A</sub> agonist and D<sub>2</sub> receptor antagonist) and eticlopride (D<sub>2</sub> receptor antagonist) can suppress graft-induced dyskinesia (GID) but are ineffective against L-DOPA-induced dyskinesia (LID) in a rat model of Parkinson's disease. However, while the effect against GID was evaluated in grafted rats, the effect against LID was investigated only in non-grafted animals. Therefore, the present study was performed to investigate whether the

graft might enhance the responsiveness of host striatum to these anti-dyskinetic drugs. If so, the same compounds should reduce LID only in grafted rats but not in non-grafted controls. Low doses of eticlopride (0.015 mg/kg), SCH23390 (0.1 mg/kg, D<sub>1</sub> receptor antagonist), and buspirone (0.3 mg/kg) substantially reduced GID. As reported before, anti-GID effect of buspirone was not prevented by a selective 5-HT<sub>1A</sub> antagonist, suggesting that its effect is independent from 5-HT<sub>1A</sub> receptor agonism. Interestingly, the three compounds also potentially reduced LID (induced by both 6 and 12 mg/kg L-DOPA) in grafted rats but were ineffective in non-grafted dyskinetic controls. Taken together, these data demonstrate that the dopamine cell grafts strikingly exacerbate the anti-dyskinetic effect of D<sub>1</sub> and D<sub>2</sub> antagonists against both GID and LID, and suggest that the anti-GID effect of buspirone in patients may be due to blockade of D<sub>2</sub> receptors.

### **Proof-of-concept: The use of human induced pluripotent stem cells (hiPSCs) to uncover a novel developmentally-based target of therapeutic lithium in bipolar disease (BPD)**

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Neuropsychiatric disorders are difficult to model not only because their non-specific multigene pattern, but also because of the subjectivity by which they are often diagnosed. BPD, a highly-lethal illness, is unique in that 50% of patients respond to lithium. Indeed, lithium-responsiveness is often pathognomonic. Critically, lithium's mechanism-of-action is unknown; however, were its targets to be identified, lithium could provide a molecular handle for discerning underlying mechanisms & deriving better treatments (lithium has unacceptable side-effects). We generated hiPSCs from lithium-responsive BPD patients from which neurons were generated. By performing differential proteomic analysis of "BPD neurons" exposed or not-exposed to lithium, we discovered a heretofore unanticipated lithium target, "CRMP2", which plays a developmental role in neurite extension, cell migration, & channel activity by virtue of its association with tau & tubulin. We determined that GSK3β & IPP2A regulate CRMP2's interaction with cytoskeleton; excessive CRMP2 phosphorylation causes neurite retraction. Li robustly reduces CRMP2 phosphorylation, validated in vivo in mouse hippocampus. Studies are ongoing supporting the prediction that CRMP2 mutation alters lithium responsiveness in mouse models of mania. Human material confirms an association between CRMP2 & BPD. We offer a strategy for merging hiPSC technology with proteomics to develop more effective neurotherapeutic drugs.

### **Influx of blood monocytes to the brain is enhanced by antioxidants**

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Grafting in Parkinson's disease involves implantation of fetal tissue into the brain where an on-going robust neuroinflammation is present. The impact of the inflammation is still elusive, and therefore this study was focused on the neuroinflammatory process in the striatal 6-hydroxydopamine (6-OHDA) model of Parkinson's disease and the effects of antioxidant treatment. It is well known that the striatal neurotoxic injection causes neuroinflammation, however, the origin of the reactive microglia is not known, and suggestions have been made that some of the inflammatory cells are bone-marrow-derived. This has been studied utilizing radiation of the bone-marrow cells and implantation of tagged cells, however, this technique opens the blood-brain barrier. In this study, superparamagnetic iron oxide (SPIO) particles were combined with MRI. The SPIO particles were i.v. injected 24 h prior to the 6-OHDA lesion was performed. The rats were scanned for T2\*-weighted images at 1 week post-lesion using a 9.4T Bruker BioSpec. T2\*-weighted images are sensitive to iron, and the images become hypointense. The results revealed no significant difference between animals that received a striatal lesion with or without SPIO particles. Giving the animals antioxidants in the form of bilberry-enriched diet resulted in a significant striatal hypointensity compared to controls. The SPIO-particles were tagged with a fluorescent marker and immunohistochemical evaluations demonstrated that the particles were found in the lesioned striatum of antioxidant-fed rats. In animals given bilberry-enriched diet, striatal dopamine regeneration was found at later time points. Taken together, antioxidants promote influx of bone-marrow-derived monocytes to the injured area, and results in dopamine regeneration.

### **A microRNA profile of reduced mir-34b/c and increased mir-592 in adult epileptic patient-derived brain cells reveals potent disease biomarker and screening tool for transplantable stem cells**

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This study reports the utility of microRNA profiling as a biomarker and therapy development for temporal lobe epilepsy (TLE). Expression profiling of microRNAs (miRNAs), confirmed by qRT-PCR, revealed that miR-34b and miR-34c were significantly up-regulated in the hippocampus and amygdala compared to the neocortex, with miR-34b/c expression highest in the amygdala. In contrast, levels of miR-592 were significantly down-regulated in the hippocampus and amygdala compared to

the neocortex. Immunocytochemical analyses provided insights into the functional role of these miRNAs, demonstrating decreased cell proliferation and differentiation in the hippocampus and amygdala compared to the neocortex of TLE patients. Stem cell grafts from the neocortex (i.e., reduced miR-34b/c but elevated miR-592 expression) survived in the amygdala, migrated to the lesioned hippocampus, and rescued hippocampal cell loss from the kainic acid-induced epilepsy in adult rats. ELISA revealed brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), and nerve growth factor (NGF) were significantly down-regulated in the human hippocampus and amygdala compared to the neocortex, while basic fibroblast growth factor (bFGF) was significantly up-regulated in the hippocampus and amygdala compared to the neocortex. These results support the use of microRNA profiling as a biomarker and a screening tool for stem cell-based therapies targeting epilepsy.

### **Generation of cortical interneuron subtypes from human pluripotent stem cells**

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Cortical interneurons are heterogeneous local inhibitory neurons playing an essential role in maintaining a balanced activity in the cortex. Dysfunctions of these cells have been associated with several neurological and psychiatric disorders, such as schizophrenia, autism and epilepsy. Patient induced pluripotent stem cell (iPSC)-derived cortical neurons offer great promise for modelling these diseases and serve as a platform for drug discovery. Furthermore, transplantation of stem cell-derived cortical interneurons may be developed as a therapy for epilepsy.

However, comparing to some other clinically relevant neuronal cell types (e.g. dopamine neurons), our ability to direct cortical interneuron differentiation from human PSCs remains limited. We showed recently that Activin promotes neuronal differentiation by inhibiting the mitogenic Sonic Hedgehog pathway while enhancing the pro-neurogenic retinoic acid signalling. In addition, Activin promotes the acquisition of a Calretinin interneuron fate by providing a caudal ganglionic eminence identity in PSC-derived neural progenitors. Extending these findings, our current work employs different combinations of morphogens to induce MGE-like progenitors, which subsequently give rise to Parvalbumin and Somatostatin expressing interneuron subtypes.

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### **Recovery neurobiology of spinal cord injury: mechanisms gleaned from peripheral neurotization studies**

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We reported that neurotization, the surgical rerouting of intact peripheral nerves originating from spinal segments rostral to the site of injury to distal targets, could markedly improve motosensory function in rats with subacute hemisection (Konya'08) or contusion SCI (Yu'13). Unlike what had been assumed to trigger recovery (i.e., motor control rerouting) our data has engendered a novel hypothesis that central neuroplasticity including adapting afferent signals via the newly rerouted peripheral nerve and propriospinal projection network to invoke locomotion pattern generator may act for neural restoration. We propose to further discuss therapeutic effect of neurotization using data produced by rat models of hemisection and contusion SCI, including a 2-steps strategy of neural rerouting plus human mesenchymal stromal stem cell (hMSC) transplantation after chronic contusion SCI. Our work, as a bench-site progress, devises a clinically feasible procedure at both localized and systemic levels to treat traumatic SCI. Neurotization, as a peripheral approach of CNS repair, has long been studied, and even sporadically applied clinically. However, lack of understanding on the mechanisms has prevented its standardized applications. Since SCI remains an unmet medical demand, successful development of this treatment may greatly reduce suffering and cost for spinal cord trauma. (supported by CIMIT-DoD).

### **Caveolin-1 alters the amyloidogenic processing of amyloid precursor protein to amyloid-beta**

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**Introduction:** The 'Amyloid Hypothesis' suggests that the build-up of (A $\beta$ ) is a primary causal event in Alzheimer's disease. A $\beta$  is cleaved from amyloid precursor protein (APP) by the  $\beta$ - and  $\gamma$ -secretases, all of which have been identified in lipid raft-regions of the plasma membrane. Caveolae are specialised forms of lipid rafts, enriched with caveolin proteins. We examined whether expression of the most ubiquitous caveolin protein, caveolin-1, could affect the processing of APP into A $\beta$ .

**Methods:** Levels of caveolin-1 in astrocytoma cells were depleted by siRNA or over-expressed by delivering constructs carrying the myc-tagged caveolin-1 gene to these cells. Cells were lysed and media collected to detect intra- and extracellular protein levels of lipid raft proteins, APP and APP metabolites including A $\beta$ .

**Results:** After caveolin-1 levels were reduced by siRNA APP levels were unaffected, however, expression levels of A $\beta$  were significantly increased. Conversely, after over-expression of caveolin-1, APP levels were significantly reduced and expression levels of A $\beta$  were unchanged.

**Conclusion:** These results suggest that at normal physiological levels, caveolin-1 has a regulatory effect on A $\beta$  and may provide a novel therapeutic target for this disease.

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### **Sedimentation of cell suspensions in large diameter cannulae: is it a problem?**

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Cell transplantation using embryonic cells has been shown to be effective in animal models of Parkinson's disease (PD) [1,2] and Huntington's disease, likewise in humans for treatment of PD [3].

A major difference between animal and human therapies is the size of the injection cannulae used. In rodent models cells are typically delivered using small cannulae with internal diameter of 0.159 mm. In humans, larger cannulae with internal diameters of 0.5–1.0 mm will be needed and the question has arisen as to how cell suspensions will behave in such needles during the long surgical procedure.

In this study, we investigate the behaviour of cell suspensions using injection cannulae of 2 different diameters. The data show that cell sedimentation occurs in both 0.5 and 0.8 mm cannulae, in both vertical and horizontal orientations severely affecting the distribution of cells in the needle in a way that would produce a very uneven distribution of cells post injection.

**Acknowledgements:** This work was funded by the EU FP7 TRANSEURO programme.

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### **Comparison of mouse and human developing striatal gene expression**

N.N. Vinh, C.M. Kelly, A. Heuer, S.V. Precious, N.D. Allen, P.J. Kemp and A.E. Rosser

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Transplantation of neural cells derived from pluripotent stem cell sources in Huntington's disease requires that they be directed towards a medium spiny striatal DAPRPP-32 positive neuronal phenotype. There are published reports of protocols to direct the differentiation of cells to an MSN phenotype that have shown success in terms of DARPP-32 readout, but limited functional effect of these cells is available to date. This would suggest that DAPRPP-32 alone is not sufficient to confirm a mature functional MSN



neuron. Thus, there is a need to both understand more about the signals required for MSN development and to identify a profile of markers that are indicative of normal MSN differentiation. To this end, we have carried out a molecular and histological comparison of human and mouse whole ganglionic eminence (WGE) development across the period during which MSNs are generated. We performed QPCR of a range of known and potential striatal differentiation markers of WGE cells between E12 and E16 in the mouse and 6–12 weeks post conception in the human, and have attempted to correlate the striatal development between the two species. We also performed *in situ* hybridization and immunohistochemical analysis of sections over a similar developmental range for both species. We carried out electrophysiological analysis of human WGE cells over the same developmental period. For the first time, this provides key information on the genetic stages of human MSN development, and highlights some key species differences.

#### **Vascularization for neural repair and stem cell transplantation by hyaluronan-peptide hydrogels**

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Stem cell therapies are shown to be promising for diseases or injuries to the central nervous system (CNS), but the effect is limited so far. One of the reasons is microenvironment after CNS injury is not optimal for cell survival, especially due to the deficiency of angiogenesis *in situ*. Reconstruction of a blood vessel network for stem cells and injured tissue may play a key role for neural regeneration in the CNS.

Here we have designed an angio-neurogenic hydrogel to promote angiogenesis and consequently to improve stem cell therapies for neural repair. The hydrogel was made by hyaluronan (HA), a main component of extracellular matrix, and linked with mimic peptides of vascular endothelial growth factor (VEGF) for providing a vascular microenvironment in injured area of the CNS. The scaffold did promote adhesion and proliferation of endothelia and neural stem cells effectively *in vitro*. Also, it evoked the receptors of VEGF and promoted the angiogenesis *in vivo*, when transplanted in injured brain.

Our results suggest that functionalized hydrogels with peptides may provide a promising microenvironment for stem cells, and it should be a new approach for regenerative therapy of the injured nervous system.

#### **The development of operant delayed matching to position for Huntington's disease mouse models**

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Operant delayed matching to position (DMTP) tasks are typically used in rats to probe spatial working memory and usually run in standard 2-lever operant "Skinner box" chambers. The DMTP task requires the animal to recall a previously made left or right response following various delay lengths, to obtain a reward.

With the development of transgene technology there is a practical need to develop these tasks for mice. As part of a larger operant test battery we aimed to achieve this, utilising the operant 9-hole box mouse chamber, with nose-poke holes replacing levers.

With no delays, C57BL/6J mice learned the DMTP operant task to >80% accuracy which decayed as a function of delay, demonstrating the viability of this task as a test of executive function for mice. The next phase of this work is to develop the reversal learning procedure, as both short-term memory and reversal learning are early disease signs in HD mice.

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#### **AAV2-mediated striatum delivery of human CDNF prevents the deterioration of midbrain dopamine neurons in parkinsonian rat and mouse model**

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Parkinson's disease (PD) is an aging-associated neurodegenerative disorder with progressive pathology involving the loss of midbrain dopaminergic neurons. Cerebral dopamine neurotrophic factor (CDNF) was recently discovered to be more selective and potent on preserving dopaminergic neurons than other known trophic factors. The present study examined the neuroprotective and functional restorative effects of CDNF overexpression in the striatum via recombinant adeno-associated virus type 2 (AAV2.CDNF) in MPTP lesioned mice and 6-hydroxydopamine (6-OHDA) injected rats. We found that bilateral striatal AAV2.CDNF injections, 2 weeks before MPTP injections in C57/BL6 mice, improved Rotarod behavior. AAV2.CDNF pre-treatment increased tyrosine hydroxylase (TH)-immunoreactivity in the striatum. Striatal delivery of AAV2.CDNF 6 weeks after 6-OHDA injections in SD rats, was able to recover behavior deficits and resulted in a significant restoration of tyrosine hydroxylase immunoreactive (TH-ir) neurons in the substantia nigra pars compacta (SNpc) and TH-ir fiber density in the striatum. Meanwhile, long-term administration of AAV2.CDNF didn't interfere TH expression in rat striatum. Our results indicate that striatal administration of AAV2.CDNF was able to provide effective neuro-protection and neuro-restoration in the nigrostriatal system and that it may be considered for future clinical applications in PD therapy.

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