

PARKINSON'S^{UK} CHANGE ATTITUDES. FIND A CURE. JOIN US.

PROGRAMME AND ABSTRACTS

Parkinson's UK Research Conference
1–2 November 2010, York, UK

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WELCOME

Welcome to the second Parkinson's UK research conference in York, and thank you for joining us in this vibrant city.

Parkinson's UK was established more than 40 years ago, and we are proud to be the largest charitable funder of Parkinson's research in the UK. We've already invested more than £45million in groundbreaking research – by 2014, that figure will have reached more than £75million.



We've also just launched our innovative research strategy, which will guide us over the next five years. In it, we've identified four key research areas that will help to drive us forward in improving treatments for Parkinson's and ultimately developing a cure for the condition. This ambitious aim is our ultimate goal.

Our first research conference, held in 2008, was a huge success. For this conference, we had even more researchers applying to attend and present their research.

This conference has two clear goals. Firstly, we want to bring the entire UK Parkinson's research community together and give you the opportunity to discuss current (and future) research, and to develop collaborations that will help move Parkinson's research forward.

Secondly, it helps to nurture and develop the next generation of Parkinson's researchers. Parkinson's UK is very keen to support skilled researchers at the early stages of their careers. That's why our Career Development Awards and PhD Studentships exist. We have more than 100 abstracts, and a significant proportion of these are from the most talented and productive young researchers working in the UK's best research groups. The UK has an excellent reputation worldwide in Parkinson's research, and our charity aims to help this to develop further in the coming years.

I hope that you will enjoy the meeting and that it gives you an opportunity to increase your knowledge of cutting-edge Parkinson's research, exchange ideas and develop exciting new collaborations.

Have a great conference!

A handwritten signature in black ink that reads "Kieran".

Dr Kieran Breen

Director of Research and Development

CONFERENCE PROGRAMME

Posters will be on display during the conference in the Oak and Minster Rooms.

Monday 1 November

9.30am	Registration	Oak Room Foyer
	Refreshments	Events Centre Bar

Session 1	Chair: Dr Oliver Bandmann	Events Centre
10am	Welcome Dr Kieran Breen, Director of Research and Development, Parkinson's UK	
10.15am	Cell and gene-therapy approaches for treatment of Parkinson's disease Dr Deniz Kirik, Lund University, Sweden	
11am	Visual symptoms in Parkinson's disease and PD dementia Dr Neil Archibald, University of Newcastle	

11.15am	Poster exhibition	Oak and Minster Rooms
	Refreshments	Events Centre Bar and Oak Room Foyer

Session 2	Chair: Dr Stephanie Cragg	Events Centre
11.45am	The role of mitochondrial metabolism and calcium homeostasis in the mechanism of neurodegeneration in PINK1 deficiency Dr Andrey Abramov, University College London	
12pm	Lysosomal dysfunction increases exosome-mediated alpha-synuclein release and transmission Dr Lydia Alvarez, University College London	
12.15pm	Differentiation of human epidermal neural crest stem cells (hEPI-NCSC) into dopaminergic neurons Professor Maya Sieber-Blum, University of Newcastle	
12.30pm	To examine the benefits and risks of deep brain stimulation for people with PD – results from the PD SURG trial Professor Adrian Williams, Queen Elizabeth Hospital, Birmingham	

1pm	Poster exhibition	Oak and Minster Rooms
	Lunch	Tempus Restaurant/Bar and Library

Monday 1 November (continued)

Session 3	Chair: Professor Sheila Kitchen	Events Centre
2.30pm	Impulse control disorders in Parkinson's disease: reward and risk Dr Valerie Voon, University of Cambridge	
3.15pm	Nicotinamide N-methyl transferase expression protects against the toxicity of mitochondrial toxins in-vitro Dr Richard Parsons, King's College London	
3.30pm	Investigating the potential benefits of gym training in Parkinson's disease Dr Ellen Poliakoff, University of Manchester	
3.45pm	Whole-body coordination when turning in response to a visual trigger Professor Ann Ashburn, University of Southampton	

4pm	Poster exhibition	Oak and Minster Rooms
	Refreshments	Events Centre Bar and Oak Room Foyer

Session 4	Chair: Dr Alex Whitworth	Events Centre
4.30pm	Parkinson's UK Brain Bank Dr David Dexter, Parkinson's UK Brain Bank at Imperial College London	
4.45pm	Induced pluripotent stem cells and dopaminergic neurons from a Parkinson's patient with triplication of α-synuclein Dr Mike Devine, University College London	
5pm	Life is not the same without synucleins: what happens with the nigrostriatal system of triple null mutant mice? Professor Vladimir L Buchman, Cardiff University	
5.15pm	A randomised control trial examining the effectiveness of an educational DVD in newly diagnosed patients with Parkinson's disease David Maskell, University of East Anglia	
5.30pm	A novel neuroprotective therapy for Parkinson's disease using a viral non-coding RNA that protects mitochondrial Complex I activity Dr Wei-Li Kuan, University of Cambridge	
5.45pm	A novel approach to imaging networks in PD patients undergoing deep brain stimulation Dr Ashwani Jha, University College London	

6pm	Free time	
6.30pm	Drinks reception and poster exhibition	Oak and Minster Rooms
8pm	Dinner	Events Centre

Tuesday 2 November

Session 5	Chair: Dr Rosemary Fricker-Gates	Events Centre
9am	From genetics to function in Parkinson's disease Dr Mark Cookson, National Institutes of Health, Bethesda	
9.45am	Exendin-4 promotes recovery of both behavioral and neurochemical deficits in a "pre-motor" hemiparkinsonian rodent model Mr Nazir Rampersaud, University of London	
10am	Investigating the function and folding of LRRK2 Dr Patrick Lewis, University College London	
10.15am	Cellular and network substrates of excessive beta oscillations in the Parkinsonian brain Dr Peter Magill, University of Oxford	

10.30am	Poster exhibition	Oak and Minster Rooms
	Refreshments	Events Centre Bar and Oak Room Foyer

Session 6	Chair: Dr Jeremy Playfer	Events Centre
11.15am	Systematic overview of randomised trials of physiotherapy in PD Miss Natalie Ives, University of Birmingham	
11.30am	Non-invasive assessment of neuropathology by deformation based morphometry in a rodent Parkinson's disease model Dr Anthony Vernon, Kings College London	
11.45am	Transcriptome profile in the mouse substantia nigra associated with Parkinson's disease neuropathology Dr Lynn Bedford, University of Nottingham	
12pm	Delivering Parkinson's studies effectively with DeNDRoN Professor David Burn, Associate Director for Parkinson's (DeNDRoN) Engaging experiences Dr Stuart Allan, University of Manchester	
12.30pm	Inhibition of Ras-GRF1 signalling in the striatum reverts motor symptoms associated to L-DOPA-induced dyskinesia Professor Riccardo Brambilla, Cardiff University	
12.45pm	Does levodopa affect progression of neuropathology in Parkinson's disease? – a clinico-pathological study Dr Laura Parkkinen, University College London	
1pm	Closing remarks and round-up, followed by lunch	

1.15pm	Lunch	Tempus Restaurant/Bar and Library
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KEYNOTE SPEAKER BIOGRAPHIES



Dr Deniz Kirik is Head of the Brain Repair and Imaging in Neural Systems (BRAINS) Unit at Lund University in Sweden, and is co-director of the Lund University Bioimaging Center.

Dr Deniz Kirik trained as a medical doctor at Hacettepe University in Ankara, Turkey. He moved to Sweden to work with Dr Anders Björklund, and received his PhD in Neuroscience at Lund University in 2001. He then obtained independent positions at Lund University, first as Assistant Professor in 2003, Associate Professor in 2005, and was promoted to Professor in 2009.

Dr Kirik has been President of the Network of European CNS transplantation and restoration, and Associate Editor of the journal *Experimental Neurology*.

Dr Kirik has more than 15 years experience in the field of cell and gene therapy, with special focus on the development of treatments for Parkinson's. He has published more than one hundred papers during this time. His work in this area has been widely recognised and highly cited. Dr Kirik is an internationally leading scientist in studies using rodent models of Parkinson's. During the last five years, he has widened his work and completed several major studies using non-human primates as well as pig models. In addition, his group has expanded to include a high-quality recombinant AAV vector production unit for functional studies in the brain. More recently, Dr Kirik has taken a leading role in the establishment of the Bioimaging Center at Lund University, which is now seen as one of the top priority areas for the institution. His recent work in bioimaging focuses on PET and MR imaging techniques, with the aim of tracking disease progression and treatment-related changes in the brain.



Dr Valarie Voon is Deputy Clinical Director of the Behavioural and Clinical Neurosciences Institute at the University of Cambridge.

Dr Voon qualified as a psychiatrist at the University of Toronto, Canada, in 2002. She then worked as psychiatric consultant to the Movement Disorders Center at the Toronto Western Hospital. In 2009, Dr Voon completed a fellowship at the National Institute of Neurological Disorders and Stroke, National Institutes of Health in Bethesda, USA, during which she concurrently completed a PhD in Neuroscience through University College London, UK. She has published widely on psychiatric aspects of movement disorders. Her research focuses on impulsive and compulsive disorders in both the general population and in Parkinson's.



Dr Mark R Cookson is a cell biologist whose research interests include the effects of mutations in the genes associated with neurodegeneration at the cellular and molecular level.

His laboratory efforts are directed at finding the underlying pathways that lead to Parkinson's and related disorders. Dr Cookson received both his BSc and PhD degrees from the University of Salford, UK, in 1991 and 1995 respectively. His postdoctoral studies included time spent at the Medical Research Council laboratories and at the University of Newcastle, UK. He joined the Mayo Clinic, Jacksonville, Florida, as an Assistant Professor in 2000 and moved to the NIA in February 2002. Within the Laboratory of Neurogenetics, Dr Cookson's group works on the effects of mutations associated with Parkinson's on protein function.

Speaker abstracts

Events Centre

S1

Cell and gene-therapy approaches for treatment of Parkinson's disease

Kirik D

Lund University

Research during the last decade created possibilities for entirely new ways to treat patients with Parkinson's disease. We have seen the initial clinical tests of several advanced treatments. Among these, cell and gene-based therapies have a special value, as these treatments are expected to provide benefits above and beyond what may be possible to achieve using standard oral medication. The basic principle behind this expectation is that these more advanced therapies would better simulate the physiological release of dopamine that is lost in the brain during the course of the disease.

Treatments that can provide regulated synthesis and release of dopamine are likely to yield to better improvement of the disease-related symptoms, while cause minimal or no side effects that are known to develop over time after e.g. L-DOPA pharmacotherapy.

This level of ambition is considered to be necessary to meet the demands both from the clinicians who wish to take treatments that would be competitive against existing therapies into clinical trials phase, and the patients' expectations to have a better quality of life. At the same time, it puts high demands on the experimental and preclinical translational research studies. The complex structure and function of the brain makes it difficult to predict the best parameters for such advanced therapies. Nevertheless, during the recent years, we have seen very encouraging developments in the clinical translation of cell and gene therapy based strategies. Although the clinical development phase is expected to take some years and might have both success and failures in its course, the future looks bright.

Visual symptoms in Parkinson's disease and PD dementia

Archibald N¹, Clarke M², Mosimann U^{3,1} and Burn D¹

¹Newcastle University ²Royal Victoria Infirmary, Newcastle upon Tyne, ³University of Bern, Switzerland

Objective: To describe the range of visual symptoms in Parkinson's disease (PD) and PD dementia (PDD) and examine potential contributory factors to their development.

Background: Visual symptoms are common in Parkinson's disease (PD) and include blurred vision and difficulty reading, double vision, visuospatial difficulties and, perhaps most strikingly, complex visual hallucinations (CVH). A variety of factors are predictive of CVH in PD, such as disease duration, cognitive decline, depression, medication usage, impaired visual acuity and sleep disorders. We recruited a mixed cohort of people with PD and PDD (n=90) and collected data on demographic, cognitive and ophthalmological factors.

Results: Visual symptoms reported significantly more commonly in the PD and PDD groups included double vision (DV), difficulty reading despite appropriate refractive correction, misjudging objects when walking in the house, freezing in narrow spaces and a variety of hallucinatory experiences. Basic measures of visual function (acuity, contrast sensitivity) were reduced in PD and PDD. Visual symptoms such as CVH, illusory misperception, feelings of presence and sensations of visual passage will be discussed in relation to factors predictive of their occurrence in the study cohort.

Conclusions: This talk will focus both on the broad range of visual symptomatology in PD and the variety of demographic, cognitive, and visual factors involved in their development.

S2

S3

The role of mitochondrial metabolism and calcium homeostasis in the mechanism of neurodegeneration in PINK1 deficiency

Gandhi S, Vaarmann A, Wood NW and Abramov AY

University College London

Objective: Parkinson's disease (PD) is a common neurodegenerative disease characterised by progressive loss of dopaminergic neurons, leading to dopamine depletion in the striatum. The mainstay of treatment is dopamine replacement therapy.

Background: We studied the effects of dopamine in PINK1 associated PD. Loss of PINK1 function causes mitochondrial dysfunction with calcium dysregulation and susceptibility to neuronal death. However, the basis for dopaminergic neuronal vulnerability in sporadic and genetic forms of PD is not clear.

Methods: We used fluorescence imaging techniques to characterize the effects of DA on intracellular $[Ca^{2+}]_c$, mitochondrial membrane potential and reactive oxygen production in primary co-cultures of neurons and astrocytes from wild-type and PINK1 knockout mouse midbrain.

Results: We demonstrate that low concentration dopamine induces cell death in PINK1-deficient cells by mitochondrial depolarisation induced by mitochondrial permeability transition pore (mPTP) opening. Dopamine-induced mPTP opening was dependent on a complex of ROS production and calcium signalling. Dopamine-induced cell death could be prevented by application of antioxidants, inhibition of ROS production, by respiratory chain substrates.

Conclusions: This data demonstrates the mechanism of dopamine toxicity in PINK1 deficient neurons, and suggests new therapeutic strategies for neuroprotection in PD.

Lysosomal dysfunction increases exosome-mediated alpha-synuclein release and transmission

S4

Alvarez-Erviti L¹, Seow Y², Schapira AH¹, Wood MJ² and Cooper JM¹

¹University College London, ²University of Oxford

Objective: To analyse the role of exosomes in alpha-synuclein release and transmission between cells and the impact of lysosomal dysfunction in this process.

Background: Alpha-synuclein aggregation plays a central role in Parkinson's disease pathology. Direct transmission of alpha-synuclein from pathologically affected to healthy unaffected neurons may be important in propagating the disease throughout the nervous system.

Methods: Exosomes were isolated from normal and alpha-synuclein (HA tagged) over-expressing SH-SY5Y cell culture medium by serial centrifugation. Exosomes were characterized by electron microscopy, cell scattering and Western blot. Exosome preparations were incubated with normal SH-SY5Y cells overnight and alpha-synuclein transmission was assessed by Western blot and immunohistochemistry.

Results: Exosomes released from alpha-synuclein over-expressing SH-SY5Y cells contained alpha-synuclein. Incubation of these exosomes with normal SH-SY5Y cells resulted in the intracellular presence of alpha-synuclein in these cells. Inhibition of lysosomal function led to an increase in the level of alpha-synuclein released in exosomes and higher levels of transmitted protein in the recipient cells.

Conclusion: Alpha-synuclein is released and transmitted by exosomes. Lysosomal dysfunction may accelerate alpha-synuclein transmission between cells.

S5

Differentiation of human epidermal neural crest stem cells (hEPI-NCSC) into dopaminergic neurons

Sieber-Blum M and Narytnyk A

Newcastle University

Objective: A protocol was developed for the ex vivo differentiation of hEPI-NCSC into dopaminergic neurons.

Background: EPI-NCSC are neural crest-derived multipotent stem cells that persist postnatally in the bulge of hair follicles (e.g. Sieber-Blum M et al, (2006) *Stem Cells* 24:2692). Our gene expression profiles at the RNA and protein levels and in vitro clonal analyses showed that equivalent cells of human origin are also neural crest-derived multipotent stem cells. Moreover, they can be expanded ex vivo into millions of stem cells (our unpublished data).

Methods: Bulges from human hair were cultured as adherent explants. Highly motile and proliferative cells emigrated from the bulge explants (see example www.youtube.com/watch?v=TB-IYIPmz9I). Expanded hEPI-NCSC were then cultured for seven days in an empirically developed neuron progenitor culture medium and subsequently differentiated for 18 days in the presence of dopaminergic patterning factors and additional reagents. Cultures were analysed by real-time polymerase chain reaction and immunocytochemistry.

Results: Genes characteristic for midbrain dopaminergic neurons, NURR1, PITX3, EN1 and LMX1b, are expressed. The dopamine biosynthetic enzymes, tyrosine hydroxylase (TH) and DOPA decarboxylase, but not dopamine- β -hydroxylase are expressed, as well as the neurotransmitter dopamine. At the protein level, $99.4 \pm 0.6\%$ of cells express neuron-specific β -III tubulin and of those $93.4 \pm 3.2\%$ express TH as well. At the current culture endpoint, $67.8 \pm 2.5\%$ of cells are NURR1 immunoreactive, whereas $95 \pm 1.9\%$ of cells are dopamine positive.

Conclusions: hEPI-NCSC can be differentiated efficiently into dopaminergic neurons, as is expected of neural crest-derived cells. hEPI-NCSC represent a population of highly pure stem cells that is easily accessible in the hairy skin. For these reasons hEPI-NCSC are attractive candidates for cell-replacement therapy in Parkinson's disease.

S6

To examine the benefits and risks of deep brain stimulation for people with PD – results from the PD SURG trial

Williams A, Rick C, Daniels J, Patel S, Ives N, Gill S, Varma TRK, Jenkinson C, Quinn N and Wheatley K

PD SURG Collaborators, University of Birmingham

Background: Deep brain stimulation (DBS) was pioneered by Benabid and his group in the early 1990s. Since then it has become widely employed. However, until recently, there have been few studies that have directly compared DBS to best medical therapy to allow the comparison of the benefits and risks of treatment options. PD SURG is the largest trial to compare DBS to best medical therapy for people with PD and so provides unique insight into these issues.

Methods: 366 people whose PD was not well controlled by medication were randomised, either to immediate DBS or to best medical therapy, with DBS deferred for 12 months. The relative benefits of DBS versus medical therapy were determined by the change in quality of life at 12 months as determined by the participants using the PDQ-39 questionnaire and by clinicians rated Unified Parkinson's Disease Rating Scale (UPDRS). The relative risks were determined by the rate of hospitalisations, excluding those for routine procedures or standard for therapy.

Results: Both the PDQ-39 and the UPDRS show a clear benefit for DBS over best medical therapy (PDQ-39 summary index difference = 4.7 points; CI: -7.6 to -1.8; $p=0.001$). This difference is likely to be meaningful to the patient. However, the DBS group also had a clear increase in the number of hospitalisations, in both surgery related and non-surgery related events.

Conclusion: There are potentially significant risks as well as benefits associated with DBS that need to be considered before deciding on a treatment option.

S7

Impulse control disorders in Parkinson's disease: reward and risk

Voon V

University of Cambridge

Background: Impulse control disorders (ICDs) occur in 13.6% of Parkinson's disease (PD) patients (DOMINION Phase I) (Weintraub et al (2010) *Arch Neurol*).

Objective: To describe (1) the results of a multicentre case control study (DOMINION Phase II) comparing PD patients with and without ICDs (Voon et al, submitted) and (2) the relationship between DA and decision making [impulsivity (Voon et al (2010) *Psychopharm*), probabilistic learning (Voon et al (2010) *Neuron*), and risk taking (Voon et al, submitted) in PD patients with and without ICDs.

Methods: 1) Matched PD patients with and without ICDs were tested using a neuropsychiatric battery. 2) ICD patients with problem gambling and compulsive shopping were compared with PD controls using cognitive tasks with combined behavioural testing, fMRI, DA manipulation and model-based reinforcement learning algorithm. Tasks included probabilistic learning (choice between two stimuli-pairs with different gain or loss probabilities), risk (choice between sure amount and gamble amount with equal expected values), and delay discounting (choice between small immediate amount and larger delayed amount).

Results: 1) 282 ICD patients were compared with 282 PD controls. ICD patients reported greater functional impairment, depressive, anxiety and obsessive compulsive symptoms, higher novelty seeking and greater choice impulsivity. Patients with multiple ICDs were younger, had greater dyskinesia scores and consumed more alcohol. Pathological gamblers and compulsive shoppers were more similar than hypersexuality and binge eating patients. 2) ICD patients on DA learned faster from gains associated with greater striatal prediction error signal. ICD patients made more risky choices and DA enhanced the effects of risk associated with decreased striatal evaluation of risk. DA increased impulsive choice and decision time in ICD patients.

Conclusions: ICDs are associated with a range of psychiatric symptoms. DA enhances a bias towards gains and risky choices along with hastening decision times leading to pathological choices.

S8

Nicotinamide N-methyltransferase expression protects against the toxicity of mitochondrial toxins in-vitro

Kadampeswaran A, Milani ZH and Parsons RB

King's College London

Objective: To investigate whether NNMT is involved in the pathogenesis of Parkinson's disease (PD).

Background: NNMT is responsible for the N-methylation of nicotinamide to 1-methylnicotinamide, and is significantly overexpressed in the brains of patients who have died of PD. There is an inverse correlation between NNMT expression and disease duration (Parsons et al (2002) *J Neuropathol Exp Neurol*; 61:111–124), suggesting a causal link. This study was designed to assess the effect of NNMT expression upon cell viability *in-vitro*.

Methods: NNMT was stably expressed in SH-SY5Y cells, which have no endogenous NNMT expression. NNMT expression was confirmed using RT-PCR, Western blotting and activity assay. Cell death was measured using LDH release. Intracellular energy production was measured using Complex I (Cxi) assay and ATP:ADP ratio. Cell morphology was assessed using quantitative image analysis of differential contrast microscopy images. Oxidative stress was measured using GSH:GSSG and lipid peroxidation. The toxicities of MPP+, rotenone, 2,4-dinitrophenol and 6-hydroxydopamine were measured using LDH release.

Results: RT-PCR, Western blotting and activity assay confirmed that SH-SY5Y cells stably transfected with the NNMT gene (S.NNMT.LP) expressed robust levels of NNMT. Cxi activity and ATP:ADP ratio was 4-fold higher in S.NNMT.LP compared to SH-SY5Y. Neurite branching increased significantly in S.NNMT.LP cells. Although GSH:GSSG did not alter, lipid peroxidation decreased by 74% in S.NNMT.LP cells. Mitochondrial toxins were significantly less toxic towards S.NNMT.LP cells than SH-SY5Y.

Conclusions: NNMT expression appears to be neuroprotective, although the mechanisms underlying this are currently under investigation. If replicated *in-vivo*, this suggests that NNMT is not involved in PD pathogenesis, but instead may be a cellular stress response to the underlying pathogenic process.

S9

Investigating the potential benefits of gym training in Parkinson's disease

Poliakoff E¹, Galpin AJ², McDonald K¹, Kellett M¹, Dick JPR¹, Hayes S³ and Wearden AJ¹

¹University of Manchester, ²University of Salford, ³Bolton Arena

Objective: To evaluate the acceptability and effectiveness of a 10-week gym-training programme in improving reaction time, motor function and wellbeing in Parkinson's disease (PD).

Background: Physiotherapy has been shown to provide benefits for patients with PD, but there is little evidence about the potential impact of group gym-training.

Methods: Thirty-two adults with mild to moderate PD (Hoehn & Yahr stage II-III; 11 women, mean age 65.2 years, mean illness duration six years) were recruited. After stratification by illness severity, participants were randomised to either an immediate 20-week bi-weekly gym training programme at a local leisure complex, or a 10-week programme starting 10 weeks after baseline assessment. Assessments at baseline (T1), 10 weeks (T2) and 20 weeks (T3) included computerised reaction time measures; videotaped motor performance, blind-rated; PD-related quality of life (PDQ-39); and illness perceptions (BIPQ). Experiences of the programme were assessed through questionnaire items and a focus group (N=6).

Results: Overall, UPDRS motor function score did not change significantly over time in either group. However, gym training was associated with significant improvements in reaction times and some timed tests in the immediate training group (T1-T2). The delayed group showed similar improvements following gym training (T2-T3). Focus group participants enjoyed the group, obtained social benefits, and gained in confidence, both in physical functioning and general activities. However, the questionnaire measures did not show significant improvements in subjective health ratings or illness perceptions.

Conclusions: Although benefits were not apparent in the questionnaire measures or overall UPDRS scores, our findings suggest that a 10-week gym training programme in a community setting can provide some benefits for patients with PD.

Whole-body coordination when turning in response to a visual trigger

S10

Ashburn A, Kampshoff C, Burnett M and Verheyden G

University of Southampton

Objective: To evaluate axial, whole-body coordination when turning.

Background: Clinicians describe people with Parkinson's disease (PwPD) as moving en bloc with little dissociation between head, trunk and lower limbs. Restricted head movements and flexed posture can place PwPD at risk of instability and falls. Whole-body movements in PwPD have not been measured objectively before.

Methods: PwPD and healthy controls (HC) were recruited from local Parkinson's UK branches and invited to our Movement Laboratory. Latency of eye, head, shoulder, pelvis and feet movement was captured in response to a central visual trigger with an eye-tracking camera and CODAmotion. Our protocol included four tasks; looking at a target 90° from centre when sitting and standing and turning towards a target 90° and 180° from centre when standing. Participants moved towards their preferred and unpreferred side.

Results: Thirty-one PwPD (14 females, mean age 68 years, mean time with PD seven years, mean motor UPDRS score 16 points) and 15 HC (10 females, mean age 67 years) participated. Our data demonstrated a relative top-to-bottom movement pattern of axial coordination in all four tasks. Onset latencies were longer for PwPD compared with HC, and we found more significant differences for moving in the unpreferred direction. Head and shoulders did move en bloc when turning towards a target 90° and 180° from centre in standing, but surprisingly, this was also observed in our sample of HC, which suggests age and Parkinson's disease influence whole-body coordination.

Conclusion: Turning by PwPD was different compared with HC; latencies were longer and the non-preferred direction was more challenging. Turning en bloc was confirmed and suggests healthy individuals and PwPD are less prepared for environmental hazards.

Parkinson's UK Brain Bank

S11

Dexter D

Parkinson's UK Brain Bank at Imperial College London

S12

Induced pluripotent stem cells and dopaminergic neurons from a Parkinson's patient with triplication of α -synuclein

Devine MJ¹, Vodicka P^{2,3}, Thomson AJ², Houlden H¹, Burdon T², Cavaleri F², Taanman J-W¹, Schapira AH¹, Gwinn K⁴, Hardy J¹, Lewis PA¹ and Kunath T²

¹University College London ²University of Edinburgh ³Institute of Animal Physiology and Genetics, CAS, v.v.i. Czech Republic, ⁴Baylor College of Medicine, Texas

Background: α -synuclein is an instrumental player in the pathogenesis of Parkinson's disease. However, little is known about how α -synuclein biology relates to neuronal death, despite extensive studies in cell and animal models.

Objective: We sought to develop a human neuronal model of α -synuclein pathology using induced pluripotent stem (iPS) cell technology.

Methods: We generated iPS cell lines from a patient with Parkinson's disease caused by triplication of *SNCA*, which encodes α -synuclein, and an unaffected first-degree relative. We then used a monolayer neural differentiation protocol to generate midbrain dopaminergic neurons in which to analyse levels of *SNCA* mRNA and α -synuclein protein.

Results: Both sets of iPS cell lines exhibited the hallmarks of pluripotency and could be differentiated into dopaminergic neurons. A major concern with this approach is that patient-derived neurons would not show the doubling of *SNCA* expression seen in patients with triplication of *SNCA*. Since *SNCA* expression is strongly up-regulated in differentiated neurons, we first identified iPS cell lines with equivalent differentiation capacity. When we compared cell lines with similar neurogenic potential, we observed a two-fold increase in *SNCA* expression and α -synuclein protein.

Conclusions: This represents the first demonstration that dopaminergic neurons can be generated from a patient with a genetic form of Parkinson's disease. It highlights the importance of using cell lines that behave similarly under differentiation conditions, and emphasises the requirement to generate multiple-induced pluripotent stem cell clones from each patient when developing disease models.

Life is not the same without synucleins: what happens with the nigrostriatal system of triple null mutant mice?

S13

Buchman VL¹, Peters O¹, Millership S¹, Connor-Robson N¹, Kooner G¹, Anwar S², Doig N², Threlfell S², Deacon R², Bannerman D², Bolam JP², Cragg SJ², Wade-Martins R² and Ninkina N^{1,3}

¹Cardiff University, ²University of Oxford, ³Institute of Physiologically Active Compounds RAS, Moscow Region

Background and Objectives: The failure of neurotransmission in dopaminergic synapses is the central event in pathogenesis of Parkinson's disease. Therefore, functional studies of proteins involved in this process are immensely important for understanding the disease mechanism. Synucleins are intrinsic and abundant components of the presynaptic terminals of vertebrates neurons, but their exact function is unclear. Previous in vivo studies of the physiological function of synucleins were complicated by a high degree of functional redundancy within this protein family. To overcome this problem, we produced mice carrying inactivating mutations in all three synuclein genes.

Methods: Mutant mice were assessed in multiple behaviour tests, with and without pharmacological challenging of the dopaminergic system. Morphology of dopaminergic synapses and various biochemical parameters were assessed in the striatum of adult and ageing animals. The number of dopaminergic neurons in the midbrain structures and sensitivity of these neurons to toxic insults were evaluated.

Results: Triple synuclein null mutant mice demonstrated substantially compromised performance in several behavioural tests and modified response to drugs that affect survival of dopaminergic neurons or function of their synapses. Although no significant structural changes of striatal dopaminergic synapses or expression of specific protein markers were found, dopamine level in the dorsal striatum, as well as evoked dopamine release by synapses of SNpc neurons of triple synuclein null mutant mice, were substantially altered.

Conclusions: Our study revealed the role of synucleins in modulation of dopamine release by presynaptic terminals of SNpc neurons.

S14

A randomised control trial examining the effectiveness of an educational DVD in newly diagnosed patients with Parkinson's disease

Maskell D and Worth P

Norfolk and Norwich University Hospital

Objectives

- 1) To establish the effectiveness of the *Being there* DVD in educating newly diagnosed patients with PD.
- 2) To determine whether improved patient knowledge in patients with PD influences medication concordance and health-related quality of life

Background

In 2007, Parkinson's UK released the *Being there* DVD, a resource designed to educate PD patients on various aspects of the disease. As of yet, there is no evidence demonstrating the effectiveness of this resource in educating patients or how it may affect patient outcomes.

Methods: 20 patients with newly diagnosed PD underwent stratified randomisation to one of two main groups: the control group (n=10) were given verbal information at diagnosis and access to standard written information, in accordance with current best practice. The intervention group (n=10) received in addition the 'Being There' DVD. All participants completed baseline and follow up (three months) questionnaires assessing knowledge, quality of life and medication concordance. Multiple statistical methods were used appropriately to compare the two groups.

Results: Immediately post-intervention, patients in the intervention group scored on average 6.8 percent higher on the PKQ than the control group (p=0.16), this difference increased to 7.7 percent at follow up (p=0.01). Continuation of a normal lifestyle was seen to be the most important message from the DVD, which overall proved very popular with patients. Small improvement in HRQoL and concordance were observed, but did not show significance.

Conclusion: The findings of this RCT suggest the *Being there* DVD is moderately effective in increasing patient knowledge on PD. Its influence on concordance and health-related quality remain unclear, but larger studies using more participants and longer follow-up will give us a clearer idea as to the true effectiveness of such resources.

S15

A novel neuroprotective therapy for Parkinson's disease using a viral non-coding RNA that protects mitochondrial Complex I activity

Kuan W-L, Fletcher M, Poole E, Tyers P, Sinclair J and Barker R

University of Cambridge

Objective: To determine the neuroprotective function of a novel viral non-coding RNA in experimental models of Parkinson's disease (PD).

Background: PD is a neurodegenerative disorder that has as part of its core pathology the loss of nigrostriatal dopamine neurons. The aetiology of this cell loss is unknown, but does involve abnormalities of mitochondrial function. We have previously demonstrated that a human cytomegalovirus-derived non-coding RNA, p137, is effective in stabilising mitochondrial Complex I activity against neurotoxic challenge (Reeves *et al.* 2007). In addition, conjugation with a rabies viral peptide RVG-9R enables our p137 to be delivered specifically into neurons via a transvascular route (Kumar *et al.* 2007).

Methods: In this study we tested the effect of RVG-9R/p137 treatment on dopaminergic cell survival in different cell lines. Furthermore, we employed the 6-OHDA-lesioned rat model of PD, and evaluated the effect of RVG-9R/p137 on dopaminergic cell survival after intranigral or intravenous delivery, before and after lesion.

Results: We have demonstrated that the administration of RVG-9R/p137 can rescue dopaminergic cell death *in vitro* and in the 6-OHDA model of PD through a specific action on Complex I activity. Furthermore, such rescue can be achieved through intravenous delivery of this agent.

Conclusion: This therapeutic approach has major implications for the treatment of PD, especially if it has been given peripherally and can also target all cells affected by the disease process, and not just the dopaminergic nigrostriatal neurons.

S16

A novel approach to imaging networks in Parkinson's disease patients undergoing deep brain stimulation

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Objective: We use a newly described, electrophysiological method to describe cortico-subthalamic networks in humans.

Background: Both phenotype and treatment response vary in patients with Parkinson's disease. Anatomical and functional imaging studies suggest that individual symptoms may represent malfunction of different segregated networks running in parallel through the basal ganglia. Therefore, characterising such networks is a key first step in understanding the pathophysiological basis of Parkinsonian symptoms.

Methods: We performed combined resting magnetoencephalographic and subthalamic local field potential recordings in 13 patients with Parkinson's disease undergoing deep brain stimulation. Coherence was used to quantify network connectivity.

Results: Two spatially and spectrally separated resting networks were identified. A temporoparietal-brainstem network was coherent with the subthalamic nucleus in the alpha (7 – 13 Hz) band, while a predominantly frontal network was coherent in the beta (15 – 35 Hz) band. Dopaminergic medication modulated the resting beta network, by increasing beta coherence between the subthalamic region and prefrontal cortex. Subthalamic activity was predominantly led by activity in the cortex in both frequency bands.

Conclusion: The cortical topography and frequencies involved in the alpha and beta networks suggest that these networks may be involved in attentional and executive, particularly motor planning, processes respectively.

From genetics to function in Parkinson's disease

S17

Cookson M R

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In the past 15 years, we have seen an explosion in the number of discoveries identifying genetic causes of Parkinson's disease. The aim of this talk is to review first what we have learned from studies in a number of laboratories about the genetics of PD. Then, I will review work that my laboratory has been more directly involved in, in trying to understand how genetic variation might lead to PD, and what we might be able to do to intervene.

It is perhaps surprising that genetics explain a large proportion of the risk for a disorder that was considered to have no genetic basis, but several quite simple reasons explain this. First, there are many different genes for PD and related disorders that have different patterns of inheritance and different frequencies throughout the world. Second, while the risk of sporadic PD has a genetic component again, there are many genes each with relatively modest effect. I will review some of the key discoveries in rare familial disease and in risk for sporadic PD I will make the argument that these two sets of results are likely to be linked in that many of the genetic pathways are related between familial and sporadic PD.

All of this information on understanding why people have a given lifetime risk of PD is interesting, but ultimately would be more useful if it could be turned into therapeutic leads. While we are a long way from that stage, in the second part of the talk I will discuss work from several laboratories where targeting one gene that causes PD, Leucine-rich repeat kinase 2, helps us to both understand pathways and might give hints as to new drug targets.

S18

Exendin-4 promotes recovery of both behavioural and neurochemical deficits in a 'pre-motor' hemiparkinsonian rodent model

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The School of Pharmacy, University of London

Objective: To assess the therapeutic value of exendin-4 (EX-4) in a premotor rodent model of Parkinson's disease (PD) with selective noradrenergic lesion.

Background: PD research has focused on loss of nigrostriatal dopamine (DA) neurons. However, other brain regions are significantly affected, such as the locus coeruleus (LC), where noradrenergic (NA) cell bodies may degenerate before the appearance of characteristic PD motor symptoms. Many patients in this 'pre-motor' stage of PD suffer from comorbid emotional deficits. Using a 'pre-motor' rodent model of PD, we have studied the utility of EX-4, a glucagon-like peptide 1 receptor agonist, which is protective in rodent models of PD.

Methods: Sucrose preference, an index of anhedonia, was measured in male Wistar rats prior to injection of the NA neurotoxin 25mg/kg *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4), followed two days later by 4µg of 6-hydroxydopamine (6-OHDA) or vehicle. EX-4 was administered at 0.5µg/kg for four days and sucrose preference measured again following the last EX-4 injection. Amphetamine was then administered and ipsilateral turning monitored over a 120s. Rats were then decapitated and their brains removed for tyrosine hydroxylase (TH) staining.

Results: Rats treated with both toxins showed significantly decreased sucrose preference compared to shams and groups treated with either toxin alone or EX-4. Amphetamine-induced circling was significantly higher in rats given both toxins compared with DSP-4 or 6-OHDA alone or when EX-4 was administered. TH+ staining was reduced in the LC of DSP-4 and DSP-4 + 6-OHDA treated rats and this was reversed by EX-4.

Conclusions: EX-4 is able to promote both behavioural and neurochemical recovery in a 'pre-motor' rodent model of PD, supporting its use in the early stages of PD.

Investigating the function and folding of LRRK2

S19

Lewis PA, Dunn L, Jebelli J and Wingrove D

University College London

Objective: To examine the function of ROCO proteins

Background: Mutations in the large multidomain kinase LRRK2 are the most common genetic cause of Parkinson's disease, responsible for 1-5% of all cases of Parkinson's in the UK. Pathogenic alterations are found throughout the open reading frame of LRRK2, with a degree of clustering within its two enzymatic domains (a kinase and a GTPase). Data from our group and others has highlighted the impact of mutations in these domains as dysregulating their functions, although a clear unifying picture of how the function of LRRK2s function is altered in disease is yet to emerge.

Methods: In the current studies, we are using artificial mutations in LRRK2 and related members of the ROCO family of proteins to examine how the domains of LRRK2 functionally relate to each other and how familial PD mutants alter this. A key aspect of biology that we have focused on is the link between enzymatic function and complex formation, folding and protein turnover.

Results: We have data suggesting that GTP binding is a characteristic of all of the ROCO protein family members, and that this can disrupt folding and complex formation, as well as protein turnover.

Conclusions: The study of human ROCO proteins in addition to LRRK2 is a powerful approach to understand the biology of these proteins, which in turn has the potential to inform us as to the mechanisms leading to dysfunction in LRRK2 mutation carriers.

S20

Cellular and network substrates of excessive beta oscillations in the Parkinsonian brain

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Oxford University

Objective: To define how excessive brain rhythms, occurring at beta frequencies emerge in Parkinson's disease.

Background: Inappropriately synchronised brain rhythms, and especially those occurring at beta frequencies (13–30 cycles per second), often accompany movement difficulties in people with Parkinson's disease. Defining the neuronal substrates underlying excessive beta rhythms in the brain is a critical step toward devising a strategy for selectively combating these oscillations for symptomatic benefit.

Methods: We used electrophysiological and anatomical techniques in a clinically relevant rat model of Parkinson's disease to elucidate the roles played by neurons in the external globus pallidus (GP), a part of the basal ganglia, in the generation and dissemination of abnormal beta oscillations.

Results: Recording from large neuronal networks in vivo following dopamine depletion, we found that the oscillatory activity of GP neurons becomes excessively and selectively synchronised at beta frequencies, in a spatially widespread manner. The precisely timed discharges of GP neurons indicate that lateral interactions between GP neurons, and rhythmic inhibitory output to other parts of the basal ganglia, could actively support abnormal beta oscillations. To test whether structures are in place to support this possible mechanistic scheme, we recorded and labelled single GP neurons and then characterised their axonal connections. The axons of all identified GP neurons in the Parkinsonian brain target neighbouring GP neurons. Moreover, the axons of many GP neurons target every other nucleus in the basal ganglia.

Conclusions: These data suggest that GP neurons, by virtue of their activity synchronization in time and space, and their extensive axons, could orchestrate and propagate excessive beta oscillations throughout the entire basal ganglia in Parkinson's disease.

Systematic overview of randomised trials of physiotherapy in PD

S21

Ives N, Patel S, Tomlinson C, Stowe R, Meek C, Sackley C and Clarke C

University of Birmingham

Objective: To update the Cochrane review published in 2001 comparing the effectiveness of physiotherapy versus placebo or no intervention in patients with PD.

Background: Despite optimal medical and surgical therapies for PD, patients develop progressive disability. Physiotherapy is used to maximise functional ability and minimise secondary complications through movement rehabilitation within a context of education and support for the whole person.

Methods: Relevant trials of physiotherapy versus no physiotherapy (up to end of 2009) were identified by electronic searches of databases and trial registers and hand-searching of conference proceedings and scanning of reference lists. Data on walking outcomes, Timed Up and Go, gait, functional reach and UPDRS were abstracted independently by three authors, with any discrepancies resolved by discussion. Standard meta-analysis methods were used to combine data from the RCTs.

Results: 43 trials with 1898 patients were identified. Trial interventions were classified into the following comparisons: physiotherapy, exercise, treadmill, cueing, MDT rehab, dance and martial arts.

Data on the physiotherapy outcomes, velocity, Timed Up and Go, functional reach and Berg Balance Scale all significantly favoured intervention, with no evidence of heterogeneity between trials, or between the different types of physiotherapy interventions (Table 1). The UPDRS total and motor scores were also significantly improved with intervention (Table 1).

Conclusions: The updated review provides clearer evidence on the possible benefit of physiotherapy in the treatment of PD. However, the differences observed between treatments were small, and the individual studies included in this review were also relatively small. Therefore, larger placebo-controlled RCTs are needed so that the clinical effectiveness of physiotherapy can be fully established.

Table 1: Results from Meta-analysis

Outcome	Mean Difference (95% CI)
2 or 6 minute walk test (m)	14.5 (1.49 to 27.50) p=0.03
Velocity (m/s)	0.04 (0.01 to 0.06) p=0.003
Step length (m)	0.03 (0.00 to 0.06) p=0.03
TUG (s)	-0.60 (-1.04 to -0.16) p=0.008
Functional reach (cm)	2.16 (0.89 to 3.43) p=0.0008
Berg Balance Scale	3.36 (1.91 to 4.81) p<0.00001
UPDRS total	-4.46 (-7.16 to -1.75) p=0.001
UPDRS motor	-4.11 (-5.57 to -2.64) p<0.00001

S22

Non-invasive assessment of neuropathology by deformation-based morphometry in a rodent Parkinson's disease model

Vernon AC, Crum WR and Modo MM

King's College London

Objective: To apply automated morphometry analysis to magnetic resonance imaging (MRI) data acquired from a rodent model of Parkinson's disease (PD).

Background: Whole brain analysis of neuropathology by histological methods is prohibitively labour-intensive. Automated morphometry based on MRI is widely used to investigate morphological changes in healthy and disease populations in humans, using techniques such as deformation-based morphometry (DBM). Here we report the preliminary results for application of DBM to an MRI dataset acquired from a rodent model of PD.

Methods: Male Sprague-dawley rats ($n=7$) were lesioned by injection of the proteasome inhibitor lactacystin (10 μ g) into the nigrostriatal system. Controls ($n=5$) received a saline injection. MRI scans were acquired from all animals at baseline, one, three and five weeks post-surgery. All scans were linearly registered (9dof) to a canonical reference followed by non-linear high-dimensional fluid-registration¹ to compute the Jacobian determinant at each time-point. Relative differences in global volumetric scaling between groups were combined with Jacobian results to produce maps showing volume-difference between groups.

Results: DBM identified local tissue volume decreases in the ventral midbrain, striatum, cortex and lateral ventricle hypertrophy, consistent with previous data from manual MRI analysis.² Volume changes in the thalamus were also detected by DBM, which have not been previously identified.

Conclusions: DBM is a sensitive method to map changes in tissue volume in this rodent model, with the potential to reveal previously unidentified areas of abnormal neuroarchitecture. These data suggest DBM may be a useful method for non-invasive assessment of neurodegeneration in rodent models of PD.

References

- ¹ Crum et al (2005) *Phys Med Biol*; 50(21):5153–74
- ² Vernon et al (2010) *BMC Neurosci*; 11:1–18

Transcriptome profile in the mouse substantia nigra associated with Parkinson's disease neuropathology

Paine S, Mayer JR and Bedford L

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Objective: To identify transcriptome changes in the mouse substantia nigra accompanying Lewy-like inclusion formation and neurodegeneration in the context of neuronal proteasomal dysfunction.

Background: Inhibition of the ubiquitin proteasome degradation pathway is one of the major factors for the development of Parkinson's disease (Bedford L (2008) *J Neurosci*; 28(33): 8189–8198; Cook (2009) *Biochim Biophys Acta*; 1792:664–75). Identifying the underlying molecular mechanisms involved in disease progression is a major challenge and necessitates the use of relevant in vivo PD models. Following depletion of 26S proteasome function in mouse substantia nigra neurones, we reported Lewy-like inclusion formation and neurodegeneration resembling the neuropathological changes in PD. We are now investigating the molecular adaptations in our mouse model using microarray analysis.

Methods: We evaluated relative transcript changes using Ocimum 30K mouse arrays on microdissected substantia nigra and incorporating a dye swap.

Results and conclusions: We have identified a profile of transcripts that show differential abundance in the normal and 26S proteasome-depleted substantia nigra. After validation, dissemination of our results will provide novel data relating to the signal transduction pathways involved in PD: a prerequisite to the development of new therapeutic programmes targeted to slow down or prevent the death of neurones in the substantia nigra in PD.

S23

Delivering Parkinson's studies effectively with DeNDRoN

Burn D

Associate Director for Parkinson's (DeNDRoN)

S24

Engaging experiences

Allan S

University of Manchester

S25

S26

Inhibition of Ras-GRF1 signalling in the striatum reverts motor symptoms associated with L-DOPA-induced dyskinesia

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Objective: To assess whether a specific targeting of an upstream brain-specific component of the Ras-ERK pathway may provide an effective therapy for LID, we have investigated the potential role of Ras-GRF1, a specific activator of the Ras proteins in neurons of the central nervous system.

Background: L-DOPA-induced dyskinesia (LID) is a common debilitating complication of dopamine replacement therapy in Parkinson's disease (PD). Recent evidence suggests that LID may be causally linked to abnormal long-term cellular adaptations in the basal ganglia, notably the hyperactivation of the Ras-ERK signaling cascade.

Methods: We used a combination of mouse genetics, electrophysiology, behavioural pharmacology and viral vector technology.

Results: In a validated mouse model of PD, in which the LID analog abnormal involuntary movements (AIMs) can be induced by repeated L-DOPA treatment, Ras-GRF1-deficient mice were significantly resistant to the development of dyskinesia. Furthermore, in a non-human primate (NHP) model of LID, Lentiviral Vectors (LV) expressing dominant negative forms of Ras-GRF1 cause a dramatic reversion of dyskinesia severity, leaving intact the therapeutic effect of L-DOPA.

Conclusions: These data not only highlight a central role of Ras-GRF1 in governing critical aspects associated to dopamine-dependent adaptations in the striatum, but also validate the first viable therapy for LID based on intracellular signaling modulation.

S27

Does levodopa affect progression of neuropathology in Parkinson's disease? A clinico-pathological study

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Objective: To investigate the relationship between cumulative lifetime dose of levodopa (L-dopa) with nigral dopaminergic cell count and the regional distribution and load of Lewy body (LB) pathology in Parkinson's disease (PD).

Background: Several *in vitro* studies have proposed levodopa L-dopa to be toxic for dopaminergic neurons and to modulate the aggregation process of α -synuclein, but applicability of these data to patients with PD is debatable.

Methods: Ninety-six cases with well-documented clinical records relating to Parkinson's drug treatment throughout the course of their illness were identified from the archives of the Queen Square Brain Bank. Density of pigmented neurons was measured unilaterally in a single section of substantia nigra (SN) with delineation of the dorsal and ventral tiers. Cortical and nigral LB densities were determined using a morphometric approach.

Results: Mean cumulative lifetime dose of L-dopa and duration of PD correlated significantly ($p < 0.001$) and it was not possible to dissect their individual influence on SN neuronal density. A subgroup analysis of younger onset patients with a long duration of PD ($n=40$) showed no association between L-dopa and total SN neuronal density ($p=0.07$), after adjustment for age and duration of illness. In this subgroup there was, however, an inverse association between L-dopa and neuronal density in the ventral ($p=0.02$) but not in the dorsal ($p=0.27$) tier. We found no difference in the L-dopa dose between Braak PD stages ($p=0.58$) and no relationship to cortical ($p=0.58$) or nigral ($p=0.55$) LB load in the entire study population.

Conclusions: Chronic use of L-dopa in PD does not enhance progression of PD pathology.

Poster Abstracts

Oak and Minster Rooms

P1

A PINK1 mutant zebrafish model of Parkinson's disease

Flinn L, Mortiboys H and Bandmann O

University of Sheffield

Objectives: 1) To establish a stable *pink1* mutant zebrafish line and determine whether this *pink1* mutant line shares crucial characteristics with human *pink1* - mutant patients, in particular, loss of dopaminergic neurons and impaired mitochondrial function. 2) To further elucidate mechanisms leading to impaired mitochondrial function and neuronal cell death in early onset Parkinson's disease (PD).

Background: Zebrafish are a vertebrate animal model system that are highly amenable for drug screens. Autosomal recessively inherited, loss of function mutations in the *pink1* gene is one cause of early-onset PD. Zebrafish are highly amenable to high-throughput drug screens.

Methods: We screened several libraries of ENU-mutagenized fish using primers corresponding to all eight exons of *pink1* to detect any fish with a functionally relevant sequence change. *In situ* hybridisation with a probe for *tyrosine hydroxylase* (TH+) was used to stain and count dopaminergic neurons. Standard biochemical methods were used for assessment of mitochondrial respiratory chain function. Agilent gene expression chips were used to undertake hypothesis-free gene expression analysis.

Results: We found a single founder with a stop mutation in exon 7 (Y431*). Adult *pink1* -/- fish do not display any overt behavioural abnormalities. *Pink1* -/- embryos at five days post-fertilisation (dpf) have a significant ($p < 0.05$) decrease in the number of TH+ cells and reduction in mitochondrial complex I activity. 8/21 genes down-regulated in *pink1* -/- embryos are involved in energy production, mitochondrial function or oxidative stress response.

Conclusions: Our results suggest these *pink1* -/- zebrafish may be a useful tool for studying the pathogenesis of early-onset PD, and could also be used in small molecule screens to identify new drug targets.

Improved physiological BAC transgenic mouse models to understand the role of alpha-synuclein and microtubule associated protein tau in Parkinson's disease

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Objective: To develop physiologically relevant bacterial artificial chromosome (BAC)-based transgenic mouse models to better understand the role of the alpha-synuclein (*SNCA*) and microtubule associated protein tau (*MAPT*) gene loci in Parkinson's disease (PD).

Background: Genome-wide associated studies have demonstrated the key role for *SNCA* and *MAPT* in PD. Improved transgenic mouse models expressing these genomic loci are now required.

Methods: We generated several BAC transgenic mouse lines carrying wild-type or disease-associated variants of the *SNCA* and *MAPT* loci. Analysis of gene expression and functional assays of dopamine neurotransmission using fast-scan cyclic voltammetry (FCV) to assess dopamine release and re-uptake in the striatum are underway.

Results: We generated lines expressing either the wild-type or mutant (A30P) form of the 135 kb *SNCA* locus on a *Sncα* -/- background. A third line over-expressed wild-type *SNCA*. Immunohistochemical staining showed alpha-synuclein expression in dopaminergic neurons in the nigrostriatal pathway in a manner almost entirely mutually exclusive from calbindin-D28K, which is believed to label neurons resistant to degeneration. We generated six transgenic mouse lines carrying wild-type (H1 or H2) or mutant (N296H or R406W) variants of the 143 kb *MAPT* locus on a *Mapt* -/- background. Transgene expression in neurons in the hippocampus, cortex and basal ganglia was confirmed using RNA in situ hybridisation, and western blotting revealed all six human tau isoforms were expressed. Measurement of dopamine neurotransmission revealed deficits in striatal dopamine signalling in transgenic lines in the absence of overt pathology.

Conclusion: Transgenic mouse models carrying PD susceptibility loci will provide important insights into the earliest changes in PD.

P2

P3

A unique form of G-substrate in humans? Potential implications for animal models of PD

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University of Edinburgh

Objectives/Background: G-substrate was named as a specific substrate of cGMP-dependent protein kinase (Aitken *et al*, *J Biol Chem*, 1981). It is a potent inhibitor of protein phosphatase 2A (PP2A) when phosphorylated on two threonine residues.

A10 dopaminergic neurons of the substantia nigra, which have a higher level of G-substrate expression, are less vulnerable to PD toxins than the adjacent A9 DA neurons (Chung *et al*, *J Neurosci*, 2007).

Recently, an mRNA for a shorter G-substrate variant (104aa) has been described. Our database searches indicate that this variant is expressed only in humans, and not in any other mammalian species, including rodents and primates. This may have implications for the validity of these current models of PD.

Methods: We have expressed this shorter variant (which only retains one of the phosphorylatable threonine motifs) and are comparing its expression level and function as an inhibitor of PP2A.

Results: We have identified novel G-substrate interacting proteins, including E3 ubiquitin-protein ligases BRE1A and BRE1B and ubiquitin carboxyl-terminal hydrolase 8 (unpublished results). These are members of the E3 ubiquitin ligase complex, of which Parkin is a member. We will ascertain whether the new G-substrate variant has a different specificity for protein interactions. We have raised antisera to the established form of G-substrate and are raising antisera specific for this new form, to investigate potential tissue and sub-cellular localization.

Conclusions: Our studies on the potential role of this novel human-specific G-substrate variant, as well as the established form in protecting DA neurons, may lead to a better understanding of Parkinson's disease.

Mitochondrial DNA integrity in a *Drosophila* model of Parkinson's disease

Sanchez-Martinez A and Whitworth A

University of Sheffield

Objective: We aim to determine how PINK1 and parkin may act to prevent accumulation of mitochondrial damage, including mtDNA damage, recently shown to be prevalent in PD-affected neurons.

Background: The maintenance of mitochondrial integrity is crucial for neuronal signalling, plasticity and long-term survival. The structure and function of the mitochondrial network is regulated by biogenesis, fission and fusion, transport and degradation. Mutations or alterations in the expression of the factors involved in these processes are associated with various neurodegenerative disorders. In Parkinson's disease (PD), mitochondrial dysfunction and oxidative stress play a key role in the development of neuropathology. The recessive Parkinsonism-linked genes *PTEN-induced kinase 1 (PINK1)* and *parkin* maintain mitochondrial integrity by regulating different aspects of mitochondrial function, including membrane potential, cristae structure, respiratory activity and calcium homeostasis. Parkin is crucial for autophagy-dependent clearance of dysfunctional mitochondria acting downstream of PINK1 in the same pathway. PINK1 is a ubiquitously expressed serine/threonine kinase with a sort N-terminal mitochondrial targeting sequence that directs import of PINK1 into mitochondria, but it has also been detected in the cytosol. Genetic studies in *Drosophila* suggest that the PINK1-parkin pathway may act to promote mitochondrial fission, or alternatively inhibit their fusion, helping to segregate damaged mitochondria prior to autophagy. The mechanism by which parkin is activated through PINK1 in response to a mitochondrial dysfunction remains unclear.

Methods: Here we use *Drosophila melanogaster* in order to characterize the role of PINK1 and parkin in the maintenance of mitochondrial homeostasis, using a biochemical and genetics approach.

P4

P5

Behavioural characterisation of terminal and fully lesioned unilateral 6-OHDA mouse models of Parkinson's disease

Smith GA, Heuer A, Lane EL, Lelos MJ and Dunnett SB

Cardiff University

Objective: To correlate dopaminergic cell loss in the substantia nigra and terminal loss in the striatum, with behavioural correlates in a wide range of available mouse tests. It is hoped that standardisation of the lesioning technique, and a full comprehensive screen of available mouse tests will aid future studies using this model.

Background: Behavioural tests indicating the presence of complete or partial lesions in the unilaterally lesioned 6-OHDA mouse models have shown conflicting results in comparison to the 'Gold standard' rat model. Lesion success and survival rates are often lower.

To date, nigral cell loss has been correlated with amphetamine-induced rotations and cylinder, corridor and rotarod tests in the mouse.

Methods: 90 B1/6 mice were injected stereotaxically with 6-OHDA directed to the MFB, striatum or substantia nigra, and animals were weighed and received daily postoperative care for two weeks. One month following the surgery, lesioned and non-lesioned control animal groups were tested by the following: amphetamine- and apomorphine-induced rotation, spontaneous rotation, corridor, staircase, forelimb stepping, gait analysis, cylinder rotarod, balance beam, inverted cage lid, and measured for difference in activity.

Results: Survival and body weight were highest in Striatum>Nigra>MFB, compared to unlesioned controls. Group differences in all lesioned groups compared to controls were observed in spontaneous rotation, apomorphine- and amphetamine-induced rotation, rotarod, cylinder and corridor tests. A smaller bias was seen in the beam, staircase and forelimb stepping tests.

Conclusions: There are a number of quick definitive tests that can be used to show lesion success and these do not necessarily correlate to results seen in similar rat hand tests. Hand tests to check for the extent of the lesion must be carefully selected to show maximum deficit for that lesion type and numbers must be appropriately chosen to take into account survival rates and post-op care needs.

P6

Graft-induced dyskinesia in the transplanted hemi-Parkinsonian rat: pharmacological manipulation and relation to dopamine receptor levels

Smith GA, Dunnett SB and Lane EL

Cardiff University

Objective: The mechanistic similarity between graft-induced dyskinesia (GID) and L-dopa induced dyskinesia (LID) is currently unknown. This may be elucidated by the pharmacological modulation of GID with substances known to affect LID in rodent models. Regulators of G-protein (RGS) signalling are changed following 6-OHDA lesions and L-dopa treatment. Basal changes in RGS have not yet been looked at with respect to the graft.

Background: Clinical trials have shown that some Parkinson's patients develop dyskinesias in response to a dopaminergic graft, irrespective of L-dopa treatment. These hyperkinesias can be mimicked experimentally in rodents.

Methods: Lesioned rats received transplants of E14 ventral mesencephalon tissue into the denervated striatum. Abnormal inhibitory movements (AIMs) were initiated by 2.5mg/kg of methamphetamine, co-administered with a pharmacological challenge: yohimbine, naloxone, amantadine, SCH-22390, raclopride, nafadotride, WIN55, 212-5, MK-801, ferobam, CP94253, MTEP and IEM1460. [³H]SCH22390, [³H]raclopride, and [³H]mazindol binding assays were carried out on fresh frozen tissue sections to label D₁, D₂ receptors and dopamine transporters.

Results: AIMs decreased with SCH-22390 and raclopride, to a lesser extent with amantadine and nafadotride in the dyskinetic group, and were unchanged in non-dyskinetic animals. GID was unchanged by the other compounds.

Conclusions: The dyskinesia caused by L-dopa in lesioned models and methamphetamine in grafted models is differently modulated by pharmacological agents, indicative of alternate mechanisms of AIM development.

P7

Synaptic and neurophysiological deficits in a fly model of Parkinson's disease (PD) with reduced locomotion

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University of York

Objective: To determine the first deficits induced by the juvenile PD-related gene *parkin*.

Background: Mutations in *parkin* are associated with oxidative stress, defective muscle mitochondria and dopamine neuron death in adult flies. Its impact on juvenile, larval *Drosophila* has been unknown.

Methods: Confocal microscopy and electrophysiology of 3rd instar larval neuromuscular junctions (NMJ).

Results: *Parkin* mutants show a 51% increase in synaptic bouton count (ANOVA, $P < 0.001$) at the NMJ. This is completely rescued by global, neuronal or muscle expression of wild-type *parkin* (*Act5c*, *elav*, *G14*). Larval velocity is also less in *parkin* mutants, 64% of wild-type (ANOVA, $P < 0.001$), partially rescued by global or neuronal expression of wild-type *parkin*. The length change during peristalsis is not affected by *parkin*, but both the frequency of contractions and speed of movement are reduced, indicating bradykinesia. Suction electrode recordings of motoneuronal activity show reduced numbers of bursts in the *parkin* mutants (44%, $P < 0.05$). The resting potential of the larval body wall muscles is 10mV more positive in *parkin* mutants (ANOVA, $P < 0.001$). At all membrane potentials, the excitatory junction potentials (EJPs) are significantly less in *parkin* mutants.

Global overexpression of genes that scavenge reactive oxygen species partially rescues synaptic overgrowth phenotype, but have little impact on locomotion.

Conclusions: In this first physiological model of PD, *parkin* affects synaptic form and function both in the periphery and CNS. We suggest that it is harder to rescue locomotion than NMJ overgrowth, because locomotion depends on multiple synapses.

Acknowledgements: We thank Alex Whitworth (University of Sheffield) for fly stocks and Parkinson's UK and the BBSRC for funding.

P8

Dopamine neuron diversity in novel transgenic α -synuclein mouse models of Parkinson's disease

Janezic S, Anwar S and Wade-Martins R

University of Oxford

Objective: To investigate dopamine neuron diversity in the midbrain of novel transgenic α -synuclein mouse models of Parkinson's disease (PD).

Background: Selective cell death of dopamine neurons in the substantia nigra pars compacta (SNc) is a key pathological feature of PD. However, dopaminergic cell populations are heterogeneous and their vulnerability in PD shows a pronounced regional variability, with dopamine neurons in the ventral SNc being highly vulnerable and populations in the dorsal SNc and ventral tegmental area (VTA) being relatively spared from degeneration. This selective neurodegenerative pattern has been demonstrated in PD patients and animal neurotoxin models. Although possible differences in calcium homeostasis have been suggested, the reasons underlying the selective neuronal vulnerability have not been fully elucidated.

Methods: We developed novel α -synuclein transgenic mouse models of PD using bacterial artificial chromosome (BAC)-based technology. We have analysed midbrain dopamine neuron diversity, applying triple immunohistochemical labelling to analyse differential expression patterns of mutant α -synuclein and calcium-binding proteins.

Results: We generated novel transgenic α -synuclein mouse lines expressing the A30P point mutation or overexpressing the wild-type gene to model the disease-associated copy number mutation, using the human α -synuclein genomic locus (*SNCA*) within a 135 kb BAC insert. Mice were backcrossed onto a *Snca* null background to create the mutant *SNCA-A30P+Snca-/-* and overexpressing *SNCA-OVX+Snca-/-* lines. Immunohistochemistry reveals preferential α -synuclein expression in the highly vulnerable ventral SNc and very low expression in the dorsal, preferentially spared tier. We demonstrate that expression of the α -synuclein transgene and the calcium-binding protein calbindin- D_{28K} , is almost entirely mutually exclusive.

Conclusion: The molecular characterisation of these transgenic PD mouse models offers important insights into potential mechanisms underlying the selective vulnerability of dopaminergic subpopulations in PD.

P9

Chemical and knock-down models for Gaucher's disease: possible application in the study of Parkinson's disease

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Objective: To investigate the possible link between glucocerebrosidase (GBA) deficiency and development of Parkinson's disease (PD)

Background: Heterozygous GBA gene variants confer a high risk for sporadic PD. Gaucher's disease (GD) results from the reduced activity of the enzyme GBA that leads to a build-up of glucocerebroside. This potentially affects autophagy via lysosome dysfunction. Evidence also suggests that autophagy may be affected in PD and this may have an effect on the handling of alpha-synuclein (implicated in PD) and on that of dysfunctional mitochondria, which have also been implicated in PD. There is also evidence of oxidative stress occurring in both PD and GD. In order to probe the possible link between GD and PD, we set up a chemically-induced model in which SH-SY5Y cells were treated with a specific inhibitor of GBA, conduritol-b-epoxide (CbE).

Methods: Cells were incubated with CbE over a period of time such that GBA was constantly >95% inhibited. We then investigated a number of markers of cell function and oxidative stress.

Results: The chemically treated cells showed that as time progressed, mitochondrial function (as measured by ATP synthetic capacity) was impeded. In addition, markers (e.g. reduced aconitase activity) consistent with an increase in exposure to oxidative stress were observed.

Conclusions: Our results indicate that impairment of GBA activity can lead to biochemical effects similar to those seen in PD, but the mechanism by which this happens is open to speculation. We are currently developing a GBA knockdown cell model to complement the chemical one, and will also be showing data from these experiments.

C.elegans is a model for Parkinson's disease

P10

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Objective: We aim to use *Caenorhabditis elegans* (*C. elegans*) as the simplest possible model to discover new gene(s) involved in dopaminergic neuroprotection.

Background: Parkinson's disease (PD) is associated with a specific dopaminergic neurodegeneration. This phenotype can be recapitulated in many models, including *C.elegans* (Nass *et al*, *PNAS*, 2002), using 6-hydroxy-dopamine (6OHDA). This neurotoxin is an oxidized derivative of dopamine that might be particularly relevant to PD, as this compound has been found in brain and urine samples of PD patients.

Research on familial PD identified causative mutations in six major genes, but those are linked to a relatively low percentage of PD patients. The use of a powerful model organism might reveal more genes involved in PD.

Methods: Using forward genetics in *C.elegans*, we identified four mutants hypersensitive to 6OHDA.

Results: We backcrossed the strongest hypersensitive mutant four times and could therefore infer that its phenotype is caused by a mutation at a single locus. This mutation was mapped to chromosome X, using Single Nucleotide Polymorphisms (SNP). This region was narrowed to 2Mbp using classical 3-point fine-mapping based on visible genetic markers flanking the mutation. We could further reduce the region to 70Kbp by combining visible markers and SNP mapping. Using Comparative Genomic Hybridisation and deep sequencing, we could identify a mutation potentially causing the hypersensitivity. We are now in the process of confirming whether hypersensitivity is caused by this mutation.

Conclusion: We want to characterize this gene, and determine its epistatic relationship with genes involved in the dopamine biosynthesis. Additionally, we will screen for mutations in the orthologue of this gene in PD patients. We also aim to perform another genetic screen.

P11

Alternative splicing of AMPA receptor subunits contributes to abnormal corticostriatal plasticity in Parkinson's disease and L-DOPA-induced dyskinesia

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Objective: To evaluate the role of alternative splicing of striatal α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA) in the 6-hydroxydopamine (6-OHDA)-lesioned rat model of L-DOPA-induced dyskinesia in Parkinson's disease (PD).

Background: Abnormal corticostriatal plasticity is a key mechanism of L-DOPA-induced dyskinesia (LID) in PD. Antagonists at glutamatergic AMPARs, such as IEM 1460, reduce dyskinesia in rat and non-human primate models of PD. AMPAR function is regulated by post-transcriptional splicing of subunit mRNA to produce flip and flop isoforms. These experiments aimed to clarify the role of alternative AMPAR splicing in LID.

Methods: Male Sprague-Dawley rats received 6-OHDA (12.5 μ g) lesions to the right medial forebrain bundle. Group one received L-DOPA/benserazide (6/15 mg/kg, i.p.) or vehicle for 21 days, and were humanely killed one hour following treatment on day 22. Group two received vehicle, L-DOPA + vehicle or L-DOPA + IEM 1460 (3 mg/kg, i.p.) for 21 days, and were humanely killed 48 hours after the final drug treatment. Coronal sections of rostral striatum were processed for *in situ* hybridisation histochemistry, using oligonucleotide probes specific for the GluR1 and GluR2 subunits and their flip and flop isoforms.

Results: L-DOPA treatment increased GluR2-flip mRNA expression in the lesioned striatum of both groups. This was blocked by the Ca^{2+} -permeable AMPAR antagonist IEM 1460. GluR1-flip expression was increased after 48 hours drug washout, but not in acute LID. There were no changes in expression of flop isoforms.

Conclusions: Alternative splicing of AMPAR subunits contributes to abnormal striatal plasticity in LID. Increases in GluR2-flip expression depend on activation of Ca^{2+} -permeable AMPARs, which are a potential target of anti-dyskinetic therapies.

Role of the Ras-ERK signalling cascade in the direct and indirect striatal pathways

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P12

Objective: The objective of the present study is to investigate the contribution of the Ras-ERK signalling pathway in synaptic plasticity of the two main striatal neuronal populations.

Background: Medium spiny neurons are the main population of striatal neurons, processing the cortical information and conveying it to the basal ganglia output nuclei through two functionally and anatomically distinct pathways. One projects directly to the final output (direct pathway) and the other sends information via subthalamic nucleus (indirect pathway).

Striatal synaptic plasticity is vital for many forms of neuronal information storage and is disrupted in many pathological conditions, including Parkinson's disease (PD) and levodopa-induced dyskinesia (LID), a severe condition associated to the standard PD medication. It has been suggested that the two different types of neurons contribute differentially to the physiopathology of these striatal disorders. The Ras-ERK signalling cascade is an important element regulating striatal plasticity and found hyperactivated in the striata of the animals affected by LID.

Methods: To analyse the synaptic properties of these two classes of MSN, we used patch clamp techniques in brain slices from bacterial artificial chromosome (BAC) transgenic mice that confer striatal cell-type-specific expression of green fluorescent protein (GFP) in specific mutant mice for neuronal components of the Ras-ERK pathway.

Results: We have found that distinct genetic manipulations in the Ras-ERK cascade specifically affect forms of synaptic plasticity in two subpopulations of striatal medium spiny neurons.

Conclusion: A better understanding of the involvement of ERK pathway in the cellular mechanisms of striatal plasticity may lead to a better design of therapeutic interventions for L-DOPA induced dyskinesia.

P13

Expression of putative LRRK2 substrates in G2019S mutated PD brains

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Objective: To investigate the expression of putative LRRK2 phosphorylation targets, alpha-synuclein and 4E-BP1 in G2019S LRRK2 mutation and in idiopathic PD (IPD) cases.

Background: *LRRK2* gene mutations are causative of both familial and IPD. Several different mutations have been identified in the *LRRK2* gene, of which the *G2019S* mutation is most prevalent. Neuropathologically, dopaminergic cell loss in the substantia nigra is the only common feature among the *LRRK2* mutations, with some cases featuring Lewy bodies (LBs) and Lewy neurites (LNs), while others showing presence of PSP-like tau inclusions, TDP-43 inclusions or ubiquitin only inclusions. *LRRK2* is a large multi-domain protein possessing GTPase and kinase domains. *G2019S* mutation is in the kinase domain and is thought to influence kinase activity *in vitro*. Whether or not *LRRK2* functions as a protein kinase *in vivo* is not known, therefore the hunt for biological and pathophysiological substrates from human brain is a critical endeavour.

Methods: Standard immunohistochemistry (IH) and immunoblotting (IB) techniques were used on human brain tissue obtained from The Queen Square Brain Bank.

Results: Several LBs were immunopositive for alpha-synuclein-phospho-Ser129 (α -synP129) by IH in both IPD and *G2019S* mutations. Abundant α -synP129 immunopositive cortical LBs were also observed. Numerous LNs were also immunopositive for α -synP129. By IB, we detected phosphorylated forms of alpha-synuclein in frontal cortex and striatum. Variable labelling of LBs were observed with 4E-BP1 Phospho-ser65 (4E-BP1P65) antibody. Some LNs were also immunopositive for 4E-BP1P65 in both idiopathic and *G2019S* mutation *LRRK2* cases. Presence of 4E-BP1P65 was also detected in both TBS and SDS-soluble fractions in all cases with higher expression levels in *G2019S* cases compared to IPD and control cases.

Conclusions: IPD and *G2019S* mutation cases have very similar expression patterns for phospho-alpha-synuclein and phospho-4E-BP1 proteins. Some biochemical alterations were noted in *G2019S* *LRRK2* mutation cases for 4E-BP1P65 protein levels.

P14

Region-specific alterations in dopamine synapse function in mice lacking all three members of the synuclein family

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Objective: To investigate the behavioural phenotype and region-specific alterations of dopamine signalling kinetics in mice lacking all three members of the synuclein family (triple-null mice).

Background: The synucleins are conserved presynaptic proteins of poorly understood function. Alpha-synuclein is intimately involved in Parkinson's disease (PD) pathogenesis and is proposed to play key roles in regulating dopamine neurotransmission and synaptic vesicle trafficking.

Methods: We studied behavioural phenotype and dopamine neurotransmission using fast-scan cyclic voltammetry (FCV) at carbon fibre microelectrodes, to assess release and uptake of dopamine in the dorsal (caudate putamen) and ventral (nucleus accumbens) striatum in female synuclein triple-null mice.

Results: Triple-null mice showed significantly increased activity in the non-anxiogenic open-field test, a decreased rate of habituation in a novel home-cage, and increased exploratory behaviour in the hole-board test compared to wild-type controls. Analysis of dopamine signalling kinetics using FCV revealed that synuclein triple-null mice have a 1.5-fold increase in extracellular dopamine concentration after discrete electrical stimuli, specifically in the dorsal, but not ventral, striatum. Paradoxically, tissue dopamine content in synuclein triple-null mice was ~30% lower in the dorsal, but not the ventral, striatum. Structural changes in dopamine synapses were studied by electron microscopy to investigate an underlying neuroanatomical correlate of the observed increase in release.

Conclusion: Loss of all three members of the synuclein family leads to alteration in synapse function and an increase in the release probability from dopaminergic neurons specifically in the dorsal striatum, the region selectively affected in PD. This suggests synucleins act as a negative regulator of dopamine release in a region-specific manner.

P15

Differential effects of wild-type and A53T mutant isoform of alpha-synuclein on the mitochondrial proteome of differentiated SH-SY5Y cells

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Objectives: (1) To determine whether increased levels of wild-type (WT) α -synuclein (α -syn) cause changes to the mitochondrial proteome. (2) To discover if the A53T α -syn produces similar effects.

Background: Increased levels of WT α -syn, and mutant A53T α -syn, are associated with Parkinson's disease (PD). Recently, an interaction between α -syn and mitochondria has been reported. Whether this interaction has any bearing on its toxicity remains to be established.

Methods: Mitochondria were purified from differentiated human SH-SY5Y dopaminergic cells over-expressing either WT or A53T α -syn. Changes to their proteomes were investigated using 2-D difference in-gel electrophoresis (DIGE). Differentially expressed proteins were identified by LC-MS/MS. Confirmation of differential expression was performed on four proteins using Western blot analysis. Mitochondrial OXPHOS1 activities were determined using assay systems from MitoSciences.

Results: 23 mitochondrial proteins were abnormally expressed as a result of over-expression of WT α -syn. Ingenuity Pathway Analysis indicated that 13 of the over-expressed proteins were cytoskeletal, suggesting an increased interaction of mitochondria with the cytoskeletal network. A significant reduction in OXPHOS1 activity was observed in the WT α -syn cells, suggesting functional consequences of the observed changes in protein expression in the mitochondria. These changes were not generally observed with the A53T mutant. For more detailed analysis, see Pennington *et al* (2010) *J Proteome Research* 9, 2390–2401.

Conclusions: Over-expression of WT α -syn resulted in multiple changes to the proteome of mitochondria, most of which were not observed with mutant A53T α -syn. We are now employing SILAC proteomic analysis to study further changes to the mitochondrial proteome, and, in particular, to mitochondrial OXPHOS1 caused by WT α -syn.

Pathological inclusions are not found in NG2 cells in multiple system atrophy or progressive supranuclear palsy

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P16

Objective: To determine whether abnormal protein accumulation occurs in NG2-positive oligodendroglial precursor cells (OPCs) in MSA and PSP.

Background: The atypical parkinsonian disorders multiple system atrophy (MSA) and progressive supranuclear palsy (PSP) are characterised respectively by glial accumulation of abnormal α -synuclein or tau. In MSA, glial cytoplasmic inclusions contain fibrillar α -synuclein and in PSP, tau accumulates in oligodendroglial coiled bodies and in astrocytes giving rise to tufted-astrocytes. NG2, an integral membrane chondroitin sulphate proteoglycan, is a marker of OPCs that occur throughout the brain. However, their role in neurodegeneration is unclear.

Methods: After optimisation, NG2 immunohistochemistry (IHC) was validated by double immunofluorescence (DIF) using cell-specific markers (OLIG2, oligodendrocytes; GFAP, astrocytes; IBA-1, microglia). NG2 IHC was performed in a cohort of MSA (N=6), PSP (N=3) and disease controls (Parkinson's disease; N = 4) and analysed by confocal microscopy.

Results: NG2-positive cells were visualised in frozen tissue sections from cases with short post-mortem intervals (PMI). IHC revealed punctate NG2-positivity in cells with fine branching processes as previously reported. DIF indicated oligodendroglial lineage as NG2 cells expressed nuclear OLIG2 and were negative for astrocytic and microglial markers. α -Synuclein or tau positive inclusions in MSA or PSP respectively were not identified in NG2 cells.

Conclusions: Although sensitive to fixation and PMI successful, NG2 staining can be achieved in post-mortem human brain tissue. Pathological inclusions associated with MSA and PSP were not detected in NG2 cells. Understanding the role of NG2 cells in oligodendroglial pathologies may provide insight into potential therapeutic targets.

P17

The impact of lysosomal dysfunction upon mitophagy: relevance to Parkinson's disease pathogenesis

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Background: Parkinson's disease (PD) is characterised by the loss of dopaminergic neurones and the presence of alpha-synuclein aggregates in Lewy bodies. While mitochondrial dysfunction, oxidative stress and decreased protein degradation are important observations in PD brain tissue, it is not known how these inter-relate in the disease mechanisms. Mutations to PINK1 and Parkin cause autosomal recessive PD, and there is increasing evidence that they play an important role in mitochondrial turnover (mitophagy). Likewise, there is increasing evidence that lysosomal dysfunction may play a role in PD pathogenesis, which is highlighted by the observation that mutations to the lysosomal enzyme glucose-cerebrosidase (GBA) are important risk factors for PD.

Objective: To determine the impact of lysosomal dysfunction upon mitophagy and the potential for decreased glucose cerebrosidase activity to inhibit lysosomal function, mitochondrial turnover and alpha-synuclein accumulation to identify a common pathogenic pathway in PD.

Methods: To determine whether a prolonged decrease in GBA activity leads to lysosomal dysfunction, we have treated dopaminergic cell models with the GBA inhibitor condurititol-B-epoxide (CBE) for 30 days. In these models, we have studied lysosomal function, the accumulation of alpha-synuclein, turnover of damaged mitochondria and subsequent effect upon mitochondrial function and cell death. As a positive control, we have evaluated the impact of lysosomal dysfunction induced by bafilomycin and 3MA.

Results: We have demonstrated that lysosomal dysfunction leads to decreased mitophagy and alpha-synuclein accumulation. Prolonged CBE treatment resulted in decreased lysosomal function, decreased mitophagy and a transient increase in alpha-synuclein.

Conclusions: These results are important for our understanding of the role of lysosomal dysfunction in PD and its inter-relationship with alpha-synuclein aggregation, mitochondrial function and cell death.

A simple cell-based assay to follow changes to Parkin's ubiquitin-protein ligase activity

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Objectives: To design a simple assay to follow changes to Parkin's ubiquitin-protein ligase (E3) activity, that is potentially suitable for high throughput screening.

Background: Parkin is mutated in a hereditary form of Parkinson's disease, Autosomal Recessive-Juvenile Parkinsonism (AR-JP). It has recently been established that a primary function of Parkin is to target depolarised mitochondria from cells via autophagy (mitophagy), a process that is aided by PINK1.

Methods: We have developed a 96 well HEK293 inducible cell line based assay of Parkin E3 activity that follows loss of mitochondria after the addition of the mitochondrial depolariser CCCP in the presence and absence of Parkin. It uses an inducible cell line, initially designed by us for identifying Parkin-protein interactions (Davison *et al* (2009) *Proteomics* 9, 4284-4297). Additional cell lines were also generated to express ligase-defective mutant isoforms of Parkin (C289G and T240R). The effects of proteasomal inhibitors MG132 (0 - 10 μ M), epoxomicin (10 μ M) and lactacystin (10 μ M) were also tested.

Results: Loss of mitochondrial enzyme activity was observed after the induction of Parkin in the presence of CCCP, that was not observed with Parkin mutants. It was also inhibited by the addition of proteasomal inhibitors. Addition of CCCP and induction of Parkin-promoted relocation of mitochondria to the nuclear periphery of these cells, presumably for autophagic destruction as reported previously by others.

Conclusions: We have designed a simple 96 well based assay to follow changes to Parkin's E3 activity. This assay could potentially be adapted for high-throughput screening for compounds that restore the activity of mutant isoforms of Parkin or to replicate its function.

P18

P19 Investigation of nucleotide binding properties of PINK1

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Objective: To investigate the nucleotide binding property of PINK1 and further understand the effect of disease mutations.

Background: Mutations in the PINK1 gene are causative of autosomal recessive early-onset Parkinson's disease. PINK1 is the only known protein kinase primarily localised to mitochondria of cells. While PINK1 retains all the structural motifs required for nucleotide binding and catalytic activity, we have been unable to confirm kinase activity in vitro. A unique feature of its kinase domain is the presence of three insert regions interspersed between the sub-domains of the N-lobe. How these insertions influence PINK1 function remains unclear.

Methods: A large number of disease-causing mutations affect residues predicted to be critical for nucleotide binding. We have investigated whether PINK1 is able to bind nucleotides including ATP, and have investigated the effect of disease causing mutations. We have expressed PINK1 in *E.coli* and developed an assay of ATP binding using the fluorescent analogue, 2'3'-O-2,4,6 trinitrophenyl ATP (TNP-ATP).

Results and conclusions: Our preliminary results indicate that PINK1 can bind ATP, but the structural requirements for binding may be non-canonical. These findings may help to shed light on how PINK1 is activated and how disease mutations confer pathogenicity.

P20 Development of α -synuclein as a molecular biomarker in Parkinson's disease

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Objectives: We aim to use ELISA methods for the measurement of 'total', 'soluble oligomeric' and phosphorylated forms of alpha-synuclein (a-syn), in longitudinal Parkinson's disease (PD) blood plasma samples.

Background: PD is characterized pathologically by the selective loss of dopaminergic neurones in the substantia nigra of the brain and the presence of Lewy bodies in surviving cells. The major protein component of Lewy bodies is a-syn, which accumulates in a phosphorylated and aggregated form. Rare cases of familial PD are caused by missense mutations in the SNCA gene and duplication and triplication events, suggesting that the expression level of a-syn is an important determinant of PD. It is now clear that a-syn is released from cells and is present in blood plasma.

Methods: We have developed new ELISA methods for the measurement of 'total', 'soluble oligomeric' and phosphorylated forms of a-syn in human plasma, working towards the development of a useful diagnostic test. We have collaborated with DeNDRoN North West to carry out longitudinal studies on patients with PD to determine whether a-syn levels correlate with disease progression. For these studies, serial blood samples have been taken at repeat visits to the clinic over a two to three year period. Regular six-monthly samples have been collected from a cohort of 200 patients (along with 30 controls). Hoehn and Yahr and UPDRS have been used to monitor disease progression in these patients.

Results: ELISAs for the measurement of 'total' and 'soluble oligomeric' forms of a-syn, as well as the phosphorylated protein, have been developed and validated. The various forms of the protein can be detected in human plasma. Results of this study will be presented.

Conclusions: These findings will lead to an understanding as to whether a-syn can be viewed as potential biomarker for the diagnosis and/or progression of PD and related diseases.

P20

P21

Development and characterisation of viral vector models to study molecular mechanisms of Parkinson's disease

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Objective: Develop a better animal model of the molecular pathways linked to PD and explore cell-based and pharmaceutical-based therapies for treating PD.

Background: PD is a common neurodegenerative disorder. It is a slow progressing, debilitating disease that is characterised by motor abnormalities, including: tremors, slow movements, rigidity and postural instability. Impairments stem from the progressive loss of dopaminergic neurons, which begins in the substantia nigra pars compacta. Alpha-synuclein is known to be a key player in the pathogenesis of PD, as it is deleterious to dopaminergic neurons and is present in Lewy bodies. Recently, a role for MicroRNAs in the modulation of various neurodegenerative conditions has been suggested. MicroRNAs are short, non-coding RNAs, which bind to the 3' untranslated region of coding mRNAs and result in either their degradation or inhibition of their translation. Specifically, Mir-7 has been shown to repress α -synuclein expression.

Method: A Mir-7 target sequence (Mir-7T) was produced to act as a decoy or sponge, by sequestering the Mir-7 and thus preventing its inhibition of endogenous alpha-synuclein gene expression, using a lenti-viral vector.

Results: A lenti-viral vector containing the Mir-7 target sequence has been produced and has been transduced successfully into HEK293T cells. Mir-7 has been shown to bind to and knock-down the expression of the target sequence, indicating it is functional *in vitro*.

Conclusions: Based on the positive results obtained *in vitro*, we will now test our lentivirus *in vivo* to examine the effect of Mir-7 on alpha-synuclein expression.

P22

A kinome and phosphatome RNAi screen to find novel modulators of PINK1 that alter mitochondrial morphology

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Objective: To perform a cell-based RNAi screen to find novel components of the PINK1 pathway that alter mitochondrial morphology and can rescue PINK1 mutant phenotypes in *Drosophila melanogaster* (*Dm*). These genes represent putative therapeutic targets for treating Parkinson's disease (PD).

Background: Loss-of-function mutations in *Pten-induced Kinase 1* (*PINK1*) cause familial PD. Loss of PINK1 function in *Dm* causes dopaminergic neuron loss, muscle degeneration, mitochondrial defects and reduced climbing and flight ability. Previous studies have shown that *PINK1* genetically interacts with genes that control mitochondrial fusion (*OPA1* and *Mfn*) and fission (*Drp1* and *Fis1*). RNAi-mediated knock-down of *PINK1* in *Drosophila* S2R+ cells results in elongation of the mitochondrial network.

Methods: We sought to identify genetic modulators of this phenotype by performing an RNAi-based screen on a Kinome and Phosphatome library containing 700 genes. Two parallel screens were set up in (1) a *PINK1* knock-down (KD) background and (2) a wild-type (WT) background. Analysing the results enabled us to identify genes that rescued a *PINK1* KD phenotype, but showed no modification to WT morphology. *In vivo* screening involved testing RNAi mediated knock-down of hits from the cellular screen on their ability to rescue *Dm PINK1* mutant phenotypes previously mentioned.

Results: 40 hits were re-screened under more stringent conditions, resulting in 24 confirmed hits. These were screened *in vivo* for modulation of *PINK1* mutant phenotypes. RNAi mediated knock-down of six genes rescued *Dm PINK1* mutant phenotypes.

Conclusions: Six genes have been shown to rescue *PINK1* mutant phenotypes. Further investigation into these genes should elucidate the nature of their interactions.

P23

Distinct muscarinic receptor subtypes on cholinergic interneurons promote activity-dependence of dopamine transmission in dorsal versus ventral striatum

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Objective: To investigate whether mAChR regulation of dopamine (DA) signalling depends on presynaptic activity, and identify the mAChRs responsible in sensorimotor- versus limbic-associated striatum.

Background: Striatal dopamine (DA) and acetylcholine (ACh) regulate motivated behaviours and striatal plasticity. Interactions between these neurotransmitters may be important, through synchronous changes in parent neuron activities and reciprocal presynaptic regulation of neurotransmitter release. How striatal muscarinic receptors (mAChRs) regulate DA signalling is unresolved. Reports implicate facilitation, inhibition, and several mAChR-subtypes on a variety of neurons.

Methods: We detected DA in real-time at carbon-fibre microelectrodes in mouse striatal slices.

Results: Broad-spectrum mAChR agonists (oxotremorine-M, APET) decreased DA release, evoked by low-frequency stimuli (<10 Hz), but increased the sensitivity of DA release to presynaptic activity, enhancing release by high frequencies (>25 Hz). These bidirectional effects depended upon ACh input from cholinergic interneurons to presynaptic nicotinic receptors on dopamine terminals, but not upon GABA- or glutamate-input. In caudate-putamen (CPu), knockout of M₂- or M₄-mAChRs (not M₅) prevented mAChR function indicating both M₂- and M₄-mAChRs are required. In nucleus accumbens (NAc) core or shell, mAChR control of DA release was prevented in M₄-knockouts, but not M₂- or M₅-knockouts.

Conclusions: Muscarinic receptors on cholinergic interneurons offer variable control of DA release probability, promoting how DA release reflects presynaptic activity in dopaminergic axons. Furthermore, different coupling of striatal M₂-/M₄-mAChRs to the control of DA release in CPu versus NAc suggests targets to influence DA/ACh function differentially between striatal domains. This data has recently been published (Threlfell et al, 2010, *J Neurosci*, 30:3398).

Investigating microglial activation in zebrafish models of Parkinson's disease

P24

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Objectives: 1) Determine whether exposure to the PD neurotoxin MPTP results in microglial activation in zebrafish wild-type embryos, comparable to other model systems and human patients ('proof of principle data'). 2) To assess whether there is microglial activation in a mutant zebrafish line carrying a stop mutation (Y431X) in the zebrafish homologue of the PD gene PINK1.

Background: Inflammatory mechanisms, including microglial activation, have been implicated in the pathogenesis of Parkinson's disease (PD), but it is unknown whether this is only a late and thus less relevant mechanism, or whether microglial activation may occur early in the pathogenesis of PD. Furthermore, only little is known about the role of inflammatory mechanisms in the pathogenesis of monogenically inherited PD. Zebrafish embryos are transparent and thus ideally suited to study microglial activation *in vivo*.

Methods: Wild-type zebrafish larvae (48hpf) were exposed to MPP+ for 24h, microglia were then stained using neutral red or an *in situ* probe for apoE. The number of activated microglial cells was also compared in zebrafish homozygous for the premature stop mutation in PINK1 to wild-type control fish using similar methods.

Results: A marked increase in microglial activation was observed following MPP+ exposure, suggesting the preservation of crucial pathogenic mechanisms across species barriers. Furthermore, marked microglial activation was observed in PINK1^{-/-} larvae. We are currently investigating whether inactivation of microglia may be neuro-protective for dopaminergic neurons in PINK1 mutant zebrafish embryos.

Conclusions: Our data demonstrates that the zebrafish may be a useful model to study the role of immune processes in the pathogenesis of Parkinson's disease.

P25

The effect of hypoxia on the culture of stem cells for the treatment of Parkinson's disease

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Objective: To determine the ability of inactivated mouse embryonic fibroblasts (iMEFs) to support the expansion of human embryonic stem cells (hESCs) under hypoxia for use in the production of dopaminergic (DA) neurons for Parkinson's disease research.

Background: Human ESCs are pluripotent cells, capable of generating a variety of cell types, including DA neurons. Hypoxic culture of hESCs mimics the *in vivo* environment of the developing embryo. Recent literature suggests that stem cells cultured and differentiated under hypoxia have a greater propensity for a DA neuronal fate than those cultured under normoxia (20% O₂) (Liu, S et al (2009) *Brain Research Bulletin*, 80:62–68). However, the majority of hESCs cultured under hypoxia rely on costly feeder-free systems. This work examined the effect of hypoxia on the more affordable iMEF system. We investigated whether the metabolic activity and viability of iMEFs was compromised under hypoxia, altering their ability to produce essential growth factors required hESC pluripotency.

Methods: Inactivated MEFs were plated at 21,000 cells/cm² and exposed to hypoxia (2% O₂) for 24 hours to 10 days. Metabolic activity was screened using the MTT assay and viability monitored using trypan blue. Human ESCs were passaged on to iMEFs and cultured under hypoxia for seven days to monitor colony expansion.

Results: There was no significant difference in the iMEF metabolic activity or viability, or the relative growth of hESC colonies under hypoxia when compared to the normoxic control.

Conclusions: Inactivated MEFs provide a suitable method of culturing hESCs under hypoxia, demonstrated by their stability in metabolic activity and viability, facilitating further studies using hESCs under hypoxia for the generation of DA neurons.

Striatal dopamine release in aged A53T- α -synuclein-overexpressing mice

P26

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Objective: To investigate dopamine release in A53T α -syn overexpressing mice.

Background: In humans, the A53T mutation in α -synuclein causes dominantly inherited Parkinson's disease. Mouse lines overexpressing human A53T α -synuclein display motor deficits and abnormal accumulation of α -syn and represent a model of early PD. Postsynaptic changes suggest age-related deficits in dopamine signalling. Since α -syn can influence vesicle release and recycling, we explored whether overexpression of human A53T α -synuclein interferes with dynamic dopamine release.

Methods: Fast-scan cyclic voltammetry was used to monitor electrically evoked release of endogenous dopamine at carbon-fibre microelectrodes in caudate-putamen (CPu) and nucleus accumbens (NAc) in acute striatal slices from 18–22 month-old mice. We used two mouse lines overexpressing human A53T under control of a prion promoter, in comparison to wild-type mice.

Results: Extracellular concentrations of dopamine ([DA]_o) evoked by discrete electrical stimuli (0.2 ms) were similar in A53T-overexpressors and wild-types, in both CPu and NAc. Furthermore, [DA]_o evoked by pulse trains at physiological frequencies (1–100 Hz) was similar between genotypes, in the presence and absence of cholinergic tone acting at nAChRs. However, following prolonged stimulation designed to deplete dopamine vesicles, preliminary observations suggest A53T mice may show deficits in recovery of release.

Conclusion: Given that [DA]_o were similar to wild-types, and there is an increase in striatal DA content, human A53T α -synuclein overexpression in mice may cause deficits in the relative releasability of dopamine, without modifying the activity dependence of release. Preliminary data suggests vesicle recluster following release may be hampered, which may have consequences for maintenance of releasable pool availability.

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P27

Functional characterisation of dopaminergic neuroblastoma cells over-expressing PINK1

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Objective: To characterise the functional regulation of PINK1 and its putative role in mitochondrial dynamics and homeostasis.

Background: The PINK1 protein comprises an N-terminal mitochondrial targeting motif, a putative transmembrane domain, a serine/threonine protein kinase catalytic domain, and a putative regulatory C-terminal region. PINK1 is thought to be primarily located in the mitochondria, but its physiological substrates and function remain to be established. Germ line mutations in the *PINK1* gene are associated with hereditary early-onset Parkinson's disease. *PINK1* mutations identified in patients are predicted to impair the kinase activity of the protein, suggesting that this activity of PINK1 is important for disease. It has been shown that neurons expressing wild-type PINK1, but not those expressing mutated PINK1, are protected against mitochondrial-mediated apoptosis. However, the mechanisms by which PINK1 exerts its cell survival functions are unknown. Recent data from mammalian and *Drosophila* model systems reveal a possible role for PINK1 in mitochondrial fission and fusion processes as well as in mitophagy.

Methods: We generated stable dopaminergic neuroblastoma SH-SY5Y cell lines over-expressing wild type or mutant PINK1. The PINK1 mutations include mutations associated with Parkinson's disease (G309D, A168P, L347P and W437X) and putative kinase dead mutations (K219A and K219M).

Results: We have characterised PINK1 expression in the stable SH-SY5Y cells generated. PINK1 mutations associated with Parkinson's disease and putative kinase dead mutant showed a decrease in ATP production, changes in mitochondrial DNA levels and mitochondrial morphology.

Conclusions: PINK1 mutations associated with Parkinson's disease and putative kinase dead mutant impaired the mitochondrial homeostasis and mitochondrial morphology.

The role of PINK1 and parkin in mitophagy

P28

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Objective: To investigate whether accumulation of dysfunctional mitochondria following loss of PINK1 expression is due to inhibition of mitophagy.

Background: Mitochondrial dysfunction and the perturbed degradation of proteins have been implicated in the pathogenesis of Parkinson's disease. Mutations in the *Parkin* (PARK2) and *PINK1* (PARK6) genes are a cause of familial PD. PINK1 is a putative kinase associated with mitochondria, while parkin is an E3-ubiquitin ligase. Loss of PINK1 expression leads to mitochondrial dysfunction, which increases with time. Parkin has been shown to be downstream of PINK1 in biochemical pathways and can also mediate the removal of damaged mitochondria by the autophagy-lysosomal pathway (also termed mitophagy).

Methods: Markers of autophagy, mitochondrial function and post-translational modification of proteins were investigated in human dopaminergic SH-SY5Y neuroblastoma cells using two models: long-term silencing of PINK1, or depolarisation of mitochondria by carbonyl cyanide 3-chloro phenylhydrazone (CCCP).

Results: Reduced flux through the autophagy-lysosome pathway was found to be coincident with inhibition of ATP synthesis following 12 days of PINK1 silencing. Over-expression of parkin in these cells restored both autophagy flux and ATP synthesis. PINK1 and parkin were also required for mitophagy in CCCP-treated cells. Ubiquitination of several mitochondrial proteins, including mitofusin 1 and mitofusin 2, were detected within three hours of CCCP treatment. These post-translational modifications were reduced following silencing of either parkin or PINK1.

Conclusions: PINK1 and parkin are involved in mitophagy, and perturbation of this pathway leads to accumulation of damaged mitochondria. This process appears to be mediated in part by the ubiquitination of the mitochondrial proteins mitofusin 1 and mitofusin 2.

P29

Parkinson's disease LRRK2 mutations are associated with mitochondrial abnormalities

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Objective: To determine the effects of the G2019S mutation on mitochondrial function.

Background: The PARK8 locus found at chromosome 12q12 contains a gene encoding the Leucine rich repeat kinase 2 mutations, which result in autosomal dominant Parkinson's disease (PD). *LRRK2* linked PD accounts for the largest portion of familial PD cases and has recently been identified as a susceptibility gene for idiopathic PD. Mitochondrial dysfunction has been linked to PD pathogenesis. In order to assess whether LRRK2 linked mutations affect normal mitochondrial processes, we have established fibroblast cultures from 14 patients harbouring LRRK2 mutations. This is the first study to report molecular changes associated with endogenous protein in primary cultures.

Methods: To assess the bioenergetics of mitochondrial metabolism in LRRK2 fibroblasts, we used oxygen and pH-sensitive phosphorescent probes to monitor mitochondrial oxygen consumption and lactate generation. To complement this work, we carried out *in vitro* ATP synthesis assays looking at ATP production through the various components of the electron transport chain. To compare mitochondrial membrane potential and rates of cellular ROS generation in our mutant and wild-type cells, single-cell analysis was performed by confocal microscopy.

Results: Our results showed a significant increase in respiration and electron transport chain capacity complemented by changes in mitochondrial membrane potential and ROS production in the presence of the G2019S mutation.

Conclusion: The G2019S mutation affects mitochondrial bioenergetics, suggesting a possible pathogenic role for LRRK2.

An RNAi-based screen to identify genes involved in PINK1/Parkin-mediated mitophagy

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Objective: To identify novel components of the PINK1/Parkin pathway, and characterise its role in mitochondrial elimination or mitophagy.

Background: The underlying causes of Parkinson's disease (PD) are not fully understood. However, research into the aetiology of rare monogenic forms offers potential insights into the mechanisms leading to common, sporadic PD. The recent discovery that two PD-linked genes, *parkin* and *PINK1*, play central roles in mitochondrial homeostasis, suggests that mitochondrial dysfunction may form the basis of the disease pathology. The ensuing challenge is to elucidate the wild-type roles of Parkin and PINK1 in order to understand how they confer cellular protection.

Recent findings show that CCCP-induced mitochondrial toxicification results in the translocation of the E3 ubiquitin ligase Parkin from the cytoplasm to a subset of dysfunctional mitochondria, in a PINK1-dependent manner. Subsequently, these mitochondria are eliminated from the cell via the autophagy pathway. Under such toxic conditions, Mitofusin, a mitochondrial GTPase with a pivotal role in mitochondrial fusion, becomes ubiquitinated in a Parkin- and PINK1-dependent manner, providing a putative mechanism through which dysfunctional mitochondria are sorted for degradation.

Methods: Using *Drosophila melanogaster* S2R+ cells, we aim to perform a genome-wide RNAi screen to identify novel modulators of the mitophagy pathway. Screen hits will be examined for a direct involvement in the PINK1/Parkin pathway using a number of complementary approaches, including *in vivo* genetic interaction studies in *Drosophila*.

Additionally, a potential role for Rhomboid-7, a mitochondrial protease thought to act upstream of PINK1 and Parkin in the mitophagy pathway, will be assessed using both cell-based and *in vivo* techniques.

Results: Latest results to be presented and discussed.

P30

P31 Glucocerebrosidase and Parkinson's

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Objectives: 1) To identify potential differences between neuronal and glial cells with regards to glucocerebrosidase (GBA) activity. 2) To document downstream metabolic events that occur as a consequence of a blockade of GBA activity.

Background: GBA deficiency is responsible for causing Gaucher disease. However, it is now recognised that homozygous mutations affecting GBA activity may convey up to a 20-fold increase risk of developing Parkinson's disease (PD). Furthermore, for heterozygote carriers, there is also a significant risk (x5) of developing PD. This observation is likely to be of importance with regards to our understanding of the pathogenesis of PD. Currently, the biochemical mechanisms involved are not known.

Methods: GBA activity was determined in human neuronal (SH-SY5Y) and astrocytic (1321N1) cell lines. β -galactosidase was also evaluated in both cell types, i.e. as a reference enzyme. GBA activity was inhibited (-90%) in neuronal cells by the use of conduritol- β -epoxide (CBE, 50 μ M for 5 days). A proteomic approach was employed to identify proteins that are differentially expressed as a result of CBE treatment.

Results: GBA activity was found to be greater in the neuronal cells (195 ± 16 vs 66 ± 0.6 nmol/h/mg, $p < 0.005$). In contrast, β -galactosidase activity was comparable in the two cell types. CBE treatment was associated with increased expression of mitochondrial malate dehydrogenase (MDH).

Conclusions: Our findings may indicate a particular importance for GBA in neuronal cells. Increased expression of MDH, following GBA inhibition, could point to a perturbation of energy metabolism and/or increased mitochondrial number.

P32 Properties of NMDA receptors in the rat substantia nigra pars compacta: antagonist inhibition and activity-dependent regulation

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Objective: Investigation of activity-dependent regulation and antagonist inhibition of NMDA receptors (NMDARs) in dopaminergic neurons of the rat substantia nigra pars compacta (SNc).

Background: NMDARs are one type of glutamate receptor and have unique functional properties, including a high calcium permeability. Pathological NMDAR over-activation, known as excitotoxicity, may contribute to degeneration of SNc DA cells in Parkinson's disease (PD). Several protective processes can limit excessive calcium influx through NMDARs, including calcium-dependent rundown, a negative feedback mechanism where elevations in cytosolic calcium activate calcium-dependent proteins that negatively modulate NMDARs. Agonist binding can also promote receptor internalisation from the cell surface, by inducing a receptor conformational change that promotes clathrin-mediated endocytosis. This effect is independent of ion flux through the receptor.

Methods: These protective mechanisms, along with pharmacological inhibition of NMDARs, were investigated in dopaminergic neurons of the SNc, using whole-cell patch-clamp electrophysiology in acutely isolated brain slices from seven day-old rats.

Results: Repeated NMDA applications give a pronounced decline in peak NMDA current. This was reduced by enhancing intracellular calcium buffering or blocking ion flux through the receptor. In order to gain insight into the subtypes of NMDARs expressed in the SNc, block by the NR2B subunit-specific antagonist ifenprodil was investigated. This revealed an incomplete inhibition consistent with a mixed population of NMDAR subunits, which may be important in future therapeutic approaches to PD.

Conclusion: Our results indicate that dopaminergic neurons of the SNc have robust mechanisms in place to negatively regulate NMDARs both in a calcium-dependent and calcium-independent manner.

Acknowledgements: AW is supported by a Parkinson's UK Scholarship.

P33 m-TOR pathway in human fibroblasts carrying LRRK2 mutation

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Objective: Investigation of m-TOR pathway in human fibroblasts expressing LRRK2 mutant protein (G2019S, Y1699C or R1441G).

Background: LRRK2 mutations are the major genetic causes of PD. LRRK2 physiological function is unknown, and so is the way that the mutations affect it. Reasonably, LRRK2-related PD could be due to a gain of toxic functions, as well as to a loss of physiological functions. Due to its enzymatic, kinase activity and to its homology with MAPKKK proteins, LRRK2 was hypothesized to be involved in signalling pathways (e.g. m-TOR).

Methods: 12 cell lines were isolated from human carriers and aged-matched controls following ethic approval (Royal Free Hospital, London) and informed consent. Controlled inactivation/re-activation of the m-TOR pathway was achieved by 1) overnight serum starvation followed by two hours of amino acid deprivation 2) 10–30 minutes amino acid stimulation of starved cells. Total cell lysates were analyzed by Western blot to quantify S6 phosphorylation. Neutral red dye was used to stain lysosomes and autophagosomes.

Results: Phosphorylation of S6 is a marker of m-TOR1 complex activity. Cell lysates were probed for p-S6. Starvation of nutrients/growth factors provoked a strong and reproducible reduction of m-TOR activity in all cell cultures, while amino acid stimulation of starved cells led to m-TOR controlled reactivation. The extent of reduction/reactivation of the m-TOR pathway was quantified and comparisons were carried out among all fibroblast cultures. Results were confirmed in an independent way by investigating variations in the content of lysosomes/autophagosomes by Neutral Red staining.

Conclusions: m-TOR has been proposed as a possible signalling pathway to host a role for LRRK2 kinase activity. Our analysis offers a general, preliminary description of the activity of m-TOR pathway in human fibroblasts carrying LRRK2 mutations.

14-3-3 isoforms in lipid rafts: the role in neurodegenerative diseases

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Objectives: Identify a link between 14-3-3 proteins and lipid raft proteins in neurodegenerative diseases.

Background: Members of the 14-3-3 protein family are involved in the protein aggregates that accumulate in specific areas of the brain in a number of neurodegenerative diseases, including Parkinson's disease (PD). 14-3-3 is sequestered into Lewy bodies by α -synuclein and binding of α -synuclein to 14-3-3 reduces protein turnover of the former, resulting in toxic accumulation.

A new emerging feature of disease pathology is the processing of proteins at lipid rafts. α -synuclein co-localises with a raft-associated protein (CD55) and also interacts with raft lipids. Other PD proteins, including the Leucine Rich Repeat Kinase 2 (LRRK2) and parkin, are also associated with lipid rafts, which indicates involvement of these membrane domains in the pathology of PD.

Methods: Lipid rafts were extracted from rat brain using 1% Triton X-100 and purified using step gradients of Optiprep solution. By employing a chloroform:methanol extraction procedure, we have been able to concentrate the proteins and remove lipids for successful Western blot analysis.

Results: We have analysed lipid raft proteins by immunoblotting with our 14-3-3 isoform specific antibodies. We have identified all five major mammalian brain forms of 14-3-3 including the two phosphoforms (Brechin et al, 2010). This was also carried out on 2D SDS-PAGE.

Conclusions: We have identified the 14-3-3 isoforms that associate with rafts, and we are currently identifying raft proteins with which 14-3-3 interacts. Preliminary results include proteins implicated in PD.

P34

P35

Reliability of different approaches used in the assessment of L-dopa and graft-induced AIMs in 6-OHDA lesioned rats

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Objective: The current study aims to establish the reliability of the different rating scales used to evaluate the severity of abnormal involuntary movements (AIMs) in the 6-OHDA lesioned rat model of Parkinson's disease (PD).

Background: At present, L-dopa is the best treatment available to treat PD. Unfortunately, after a few years of chronic treatment, most patients receiving it develop AIMs. An alternative approach to the treatment of PD could be the replacement of the lost striatal dopaminergic innervation by dopaminergic neurones derived from foetal tissue. However, despite showing benefits in some patients, there were reports of dyskinesia developing following transplantation without drug-treatment. Both forms of these behaviours, L-dopa and graft-induced dyskinesia, have been modelled in the 6-OHDA lesioned rodent and AIMs are assessed using subjective rating scales that differ between laboratories.

Methods: To establish the reliability of three different AIMs scoring methods, we used 6-OHDA unilaterally lesioned rats treated daily with L-Dopa (6mg/kg then 12 mg/kg s.c. with 15mg/kg benserazide). AIMs assessment was carried out in two environments: 1) circular rotometers or 2) rectangular cages. Animals were then tested twice a week for three months. Rats were transplanted with ventral mesencephalon from e14 embryos. 12 weeks post-transplantation, L-dopa induced behaviours were reassessed using the different protocols and were compared to graft-induced dyskinesia.

Results: Contrary to what was suggested, the shape of the environment has no impact on the severity of the rotational behaviour.

Conclusions: The scoring protocols used show different time courses during chronic L-dopa administration, but we found no difference between the two environments.

Mitochondrial dysfunction causes age-related and cell type-specific neurodegeneration in *Drosophila*

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P36

Objective: To determine whether mitochondrial dysfunction causes dopaminergic (DA) neurodegeneration in a *Drosophila* model of Parkinson's.

Background: Mitochondrial dysfunction has been associated with several major neurodegenerative disorders, including Parkinson's disease (PD). Progressive degeneration of DA neurons in the substantia nigra of the ventral midbrain remains a major pathological hallmark observed in PD. Ageing individuals with PD have high levels of mitochondrial DNA (mtDNA) alterations, and mutations in PD-related genes *PINK1* and *parkin* have been associated with reduced mtDNA copy numbers and decreased mitochondrial respiratory activity. However, it is not clear whether mitochondrial dysfunction is causally related to DA neurodegeneration.

Methods: To address the causal relationship between mitochondrial dysfunction and DA neurodegeneration *in vivo*, we used the fruitfly *Drosophila* as a model system. Genetically targeted RNA interference was used to disrupt mtDNA replication and repair and the mitochondrial electron transport chain in DA neurons as compared to cholinergic neurons. The resulting phenotypes were analysed at the molecular, cellular and behavioural level.

Results: Ageing flies show progressive locomotor defects affecting climbing and walking behaviour when mtDNA alterations and subsequent mitochondrial dysfunction is targeted to DA, but not when targeted to cholinergic neurons. The behavioural defects are accompanied by adult-onset, age-related degeneration of DA neurons. Application of nicotinamide ameliorated the locomotor defects observed in these flies. In contrast to mtDNA alterations, DA-specific mitochondrial respiration defects did not alter locomotor behaviour.

Conclusions: Our results provide experimental evidence that age-related mitochondrial dysfunction is one of the pathogenic pathways underlying PD-related dopaminergic neurodegeneration.

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P37

Deciphering the role of DJ-1 in the etiology of Parkinson's disease using yeast as a model

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Objective: To determine the function of DJ-1 using the yeast *Saccharomyces cerevisiae* as a model organism.

Background: Mutations in the gene *PARK7*, which encodes the protein DJ-1, cause early-onset cases of Parkinson's disease (PD). Though the precise function of DJ-1 is unknown, it is thought to be important in protecting the brain from oxidative stress by acting as a redox-dependent chaperone.

Methods: Yeast express four proteins that are members of the DJ-1 family: Hsp31, Hsp32, Hsp33, and Hsp34. We generated single, double, triple and quadruple knock-out strains and characterised the resulting phenotypes under different oxidative stress conditions. In order to test the regulation of the DJ-1 homologs under stress conditions, we quantified the levels of *HSP31*, *HSP32* and *HSP33* expression in the presence or absence of hydrogen peroxide by quantitative real-time PCR.

Results: Expression of these genes is upregulated in the presence of an oxidant, supporting their role in oxidative stress response. We found that the four homologs suppress toxicity in a yeast model of Huntington's disease and that deleting these genes enhances the toxicity of α -synuclein, another protein implicated in PD.

Conclusions: Our results suggest that the yeast DJ-1 homologs are functionally related to human DJ-1. This work will now enable us to use these strains as models of PD in various studies, including synthetic lethal screens that will ultimately identify genes and pathways involved in DJ-1 function.

Cerebrospinal fluid alpha-synuclein levels as a biomarker for Lewy body disease

P38

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Objective: To evaluate measures of alpha-synuclein (α -syn) within cerebrospinal fluid (CSF) as a biomarker for Lewy body disease.

Background: Parkinson's disease (PD) and dementia with Lewy bodies (DLB) are characterised pathologically by the presence of α -syn immunoreactive Lewy bodies within the cerebral cortex and substantia nigra. However, clinical diagnosis can be imprecise and there is great need for a biomarker that would aid the improvement of diagnostic accuracy.

Methods: CSF samples from 56 individuals with Lewy body disease (LBD), (39 with PD, 17 with DLB), seven with progressive supranuclear palsy (PSP), four with multisystem atrophy (MSA) and 18 healthy controls (supplied by the Parkinson's Brain Bank) were assayed for total α -syn, oligomeric α -syn, phosphorylated α -syn (pS- α -syn) and oligomeric phosphorylated α -syn (oligo-pS- α -syn) by immunoassay.

Results: Although CSF levels of total and oligomeric forms of α -syn did not vary significantly between the diagnostic groups, those for oligo-pS- α -syn did differ ($p < 0.001$), with a trend ($p = 0.045$) towards this for pS- α -syn. Although all four α -syn measures were highest in patients with MSA, only those for oligo-pS- α -syn were significantly ($p < 0.001$) greater compared to the other groups.

Conclusion: None of the present immunoassays for α -syn, based on post mortem CSF samples, could absolutely differentiate patients with PD or DLB from controls or patients with PSP. However, measures of α -syn, especially phosphorylated forms of α -syn, within CSF from living patients, earlier in the course of their illness, might prove more informative. The increases in oligo-pS- α -syn levels between MSA and PD/DLB groups may provide a test to distinguish between these synucleinopathies.

P39

Proteasome inhibition induces α -synuclein-positive aggregates in the myenteric plexus of jejunum in rats

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Objective: To investigate the effects of a proteasomal inhibitor I (PSI) on the myenteric plexus in the jejunum following PSI administration.

Background: Systemic administration of a proteasomal inhibitor I (PSI) to rats produces widespread brain pathology similar to that seen in Parkinson's disease (PD). Whether the gastrointestinal changes, in particular in the myenteric plexus as observed in PD, are also observed in this animal model remains to be determined and was the main aim of this study.

Methods: Rats (200–250g; n=6) were treated with either PSI (5.0mg/kg, s.c.) or vehicle (100% DMSO, 1.0ml/kg, s.c.) on days 1, 3, 5, 8, 10 and 12. Behavioural and histological changes were assessed nine weeks following PSI administration, using automated locomotor activity equipment and immunohistochemical analysis of tyrosine hydroxylase (TH) in the substantia nigra (SN) and α -synuclein (α -SYN) in the myenteric plexus of the jejunum.

Results: PSI administration did not alter spontaneous locomotor activity. However, a loss of TH⁺ neurones in the SN was observed. There was no cell loss in the myenteric plexus of jejunum following PSI administration, although morphological changes at cellular levels were observed. Importantly, α -SYN formed aggregated-like structures in the myenteric plexus following PSI administration.

Conclusions: PSI treatment induced nigral dopaminergic cell death in the SN as previously reported. No cell loss was observed in the myenteric plexus of jejunum although α -SYN⁺ aggregates were formed, suggesting damage to the nerves of the enteric nervous system. These data suggest that the PSI rat model reflects both the brain and gastrointestinal pathology seen in PD.

P40

Expression and function of novel signalling proteins in the developing midbrain

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Objective: To analyse the expression patterns and developmental influence of candidate novel signalling proteins expressed in the developing midbrain, as identified by proteomics.

Background: Stem cell-derived neurons are frequently cited as a potential source of dopamine neurons for future treatments of PD. It is likely that additional (as yet unknown) signalling proteins, which may also function during *in vivo* specification, are required for the differentiation of stem cells to dopaminergic populations. To identify novel factors driving *in vivo* dopamine neuron development, we have performed a proteomic analysis of developing ventral mesencephalic tissue and identified several candidate proteins which may function in this capacity.

Methods: Candidate signalling proteins were selected through bioinformatic data mining of proteomic data sets. Embryonic tissue sections were cut using a cryostat and immunohistochemistry used to study protein expression in the ventral mesencephalon. Mice carrying homozygous and heterozygous mutations of specific proteins were analysed for nigro-striatal abnormalities. Primary cells were cultured in media containing proteins of interest, to investigate their influence on dopamine neuron differentiation and survival.

Results: We have identified a number of candidate signalling proteins which may play an as yet unknown role in dopaminergic neuron development, including prosaposin and otocadherin. Immunohistochemistry of embryonic tissue sections show these proteins to be expressed in a decreasing ventral to dorsal gradient within the mesencephalon. Additionally, co-labelling with tyrosine hydroxylase demonstrates expression within dopamine neurons and in areas immediately surrounding the dopaminergic region. We will report further results on the effect of these proteins on the development of dopaminergic neurons, both through the analysis of homozygous and heterozygous mutant mice, and their influence on developing primary neurons in culture.

P41

Influence of PGC-1 over-expression on a dopaminergic cell model of PINK1 silencing

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Objective: To test whether upregulation of PGC-1 is protective in Parkinson's disease (PD) pathogenesis.

Background: Peroxisome proliferator-activated receptor gamma co-activator (PGC)-1 is known as a master regulator of oxidative defence and mitochondrial biogenesis in many tissues – it increases mitochondrial mass, mitochondrial DNA transcription, expression levels of respiratory chain subunits, enhances ATP production, and mediates up-regulation of anti-oxidant enzymes. PINK1 mutations cause autosomal recessive PD and induce mitochondrial abnormalities and free radical damage.

Methods: We over-expressed and characterised PGC-1 alpha and beta in the dopaminergic SH-SY5Y cells and studied the effects of PGC-1 ectopic expression upon the influence of PINK1 silencing.

Results: We reproduced many of the features described in other PGC-1 models, including increased baseline mitochondrial function and improved free radical metabolism. Our findings indicate that PGC-1 over-expression protected SH-SY5Y cells from developing oxidative stress, but not the mitochondrial ATP synthesis defect associated with PINK1 silencing.

Conclusions: Our model of PGC-1 over-expression in dopaminergic cells shows that up-regulation of PGC-1 could protect these cells from developing the patho-biochemical features found in PD. Our work also suggests that oxidative stress and mitochondrial dysfunction developed from PINK1 silencing are two independent events.

Acknowledgements: This work is supported by Parkinson's UK, the Kattan Trust of the Royal Free hospital, and the Wellcome trust/MRC Parkinson's Disease Consortium grant.

P42

Dissecting the role of alpha-synuclein in dopamine biology using human dopaminergic cell models

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Objective: To understand the role of alpha-synuclein in dopamine homeostasis.

Background: Alpha-synuclein is central to the Lewy body neuropathology of Parkinson's disease (PD), in which cardinal motor symptoms are linked to the death of dopaminergic neurons. Recent advances suggest that alpha-synuclein plays a role in the genetics and neurodegeneration of sporadic PD. We have previously reported BE(2)-M17 as a human dopaminergic cell line that endogenously expresses tyrosine hydroxylase and (TH): takes up 3HDA in a dopamine transporter (DAT)-dependent manner: it also produces DA and releases it specifically in the presence of potassium and calcium.

Methods: We have developed two approaches to modulate alpha-synuclein expression in BE(2)-M17 cells. The first is the RNAi-mediated knockdown of alpha-synuclein. The second is the expression from BAC-SNCA-HA vectors, carrying the entire human SNCA genomic locus under the control of endogenous promoter sequences.

Results: Using RNAi we are able to suppress 80% of alpha-synuclein expression in BE(2)-M17 cells. Previously we have shown alpha-synuclein knockdown decreases DAT function, due to a reduction in DAT membrane localisation. Our current work focuses on the role of alpha-synuclein in DA synthesis and release.

Preliminary data shows that alpha-synuclein knockdown decreases intracellular DA content, with no change in TH activity. Secondly, BAC-SNCA-HA vectors have been successfully used to deliver and stably express WT or mutated alpha-synuclein in BE(2)-M17 cells at levels equivalent to endogenous alpha-synuclein.

Ongoing work seeks to use both approaches to investigate the role of alpha-synuclein in regulating DA synthesis, DA release and DAT function.

Conclusions: Our work highlights important roles for alpha-synuclein in pathways related to PD neurodegeneration. Understanding these processes is fundamental for deciphering the cellular and biological processes underlying PD aetiology.

P43

The biochemical properties of Parkinson's disease associated protein LRRK2

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Objectives: To investigate the enzymatic properties of LRRK2 using *in vitro* kinase assays.

Background: Mutations in the gene encoding LRRK2 are the most common genetic form of Parkinson's disease (PD). Relatively little is known about the function of LRRK2 and the mechanisms by which it functions. It is known that kinase activity of the protein is regulated intramolecularly by the ROC (GTPase) domain. Mutations in the ROC domain at residues R1441 and I1371 are thought to disrupt GTP hydrolysis.

It has been shown that LRRK2 autophosphorylates intramolecularly and a putative molecular target (Moesin) has been found. Kinase activity has been linked to LRRK2 toxicity, however the exact relationship between the ROC domain and kinase domain and toxicity remains unclear. Our studies focus on investigating the biochemical properties of LRRK2. This will help us to understand better the mechanisms that this protein uses to function and therefore better understand its contribution to the etiology of PD.

Methods: To investigate the role that dimerisation plays in kinase activity of LRRK2, we designed kinase assays in the presence of MBP. Recombinant LRRK2 (Invitrogen) was incubated with ^{32}P ATP for one hour. Aliquots were taken at various time points and the samples run out on an SDS PAGE gel, western blotting performed and the phosphorylation of myelin basic protein (MBP) and autophosphorylation of LRRK2 quantified.

Results: The differential impact dimerisation will be discussed with regard to the regulatory role of the kinase domain.

Mitochondrial impairment in Parkinson's disease patients with the G2019S mutation in LRRK2

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Objective: The aim of this study was to assess mitochondrial function and morphology in G2019S mutant patient tissue. This would determine whether impaired mitochondrial function and morphology are shared features in early onset and late onset PD.

Background: The G2019S mutation in the LRRK2 gene is the most common identifiable cause of PD, but the underlying mechanisms leading to neuronal cell death remain unclear. Impaired mitochondrial function and morphology have been described in different *in vivo* and *in vitro* models as well as patient tissue of early onset PD.

Methods: Skin biopsies were taken from five PD patients with the G2019S mutation. Assessment of mitochondrial membrane potential and intracellular ATP levels, as well as substrate linked mitochondrial ATP production assays, were all carried out on three independent cell preparations per patient. Results were compared to five age-matched controls. Mitochondrial elongation and interconnectivity was assessed using previously published methods.

Results: Both mitochondrial membrane potential and total intracellular ATP levels were decreased in the G2019S mutation carriers. Subsequently undertaken mitochondrial ATP production assays suggested that the observed reduction is at least partially due to impaired mitochondrial function. Mitochondrial elongation and interconnectivity were increased in the G2019S patient cohort.

Conclusions: Our results provide evidence for impaired mitochondrial function and morphology in G2019S mutant patient tissue. Further studies are required to determine whether the impaired mitochondrial function is due to increased LRRK2 kinase activity, or other mechanisms such as LRRK2 haploinsufficiency.

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P45

The role of FoxA genes in the maintenance of the midbrain dopamine neurons

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Objective: To determine whether FoxA genes have a role in the maintenance of the dopamine neurons of the ventral midbrain.

Background: The role of transcription factors in regulating the development of midbrain dopaminergic (mDA) neurons has been intensively studied due to the involvement of these neurons in neurological disorders, such as Parkinson's disease. Two members of the forkhead/ winged helix transcription factor family, Foxa1 and Foxa2, have recently been shown to be involved in the early development of mDA neurons. Here we present data demonstrating that these genes are also involved in the maintenance of the dopaminergic phenotype in mature mDA neurons.

Methods: We conditionally deleted both genes in mice. We use the Cre-*loxP* system to specifically inactivate FoxA1 and FoxA2 in mDA neurons from embryonic day 13.5 onwards, when these cells are all post-mitotic, using the dopamine transporter-Cre mouse. We performed immunohistochemical and in situ hybridization analyses on sections of midbrain from these mice at various time points after deletion and quantified the results.

Results: When these mice were examined in adulthood, the deletion of both FoxA1 and FoxA2 resulted in a 50% reduction in the number of tyrosine hydroxylase-positive mDA neurons. However, absence of cell death at any time point analyzed suggested that the 'lost' cells were not dying. Subsequent analysis with a Rosa-YFP reporter mouse line demonstrated that the 'lost' cells were still present, but not expressing any of the genes involved in dopamine synthesis.

Conclusions: These results reveal that the FoxA genes play a critical role in the maintenance of the mDA neuron phenotype.

Dmrt5 promotes the midbrain identity of ES cell-derived dopamine neurons by restricting ventrolateral and dorsal neuroepithelial fates

P46

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Objective: The aim of this study is to investigate the role of the novel transcription factor Dmrt5 in midbrain differentiation in embryonic stem cells and in the developing vertebrate embryo.

Background: The ventral mesencephalon gives rise to midbrain dopaminergic neurons, the loss of which is the primary pathology of Parkinson's disease. Understanding how the ventral midbrain is patterned, and how the different cell populations in this area are specified, is essential for the development of protocols to generate midbrain dopaminergic neurons *in vitro*.

Methods: We employ genetic-based gain- and loss-of-function embryonic stem cell (ESC) models and monolayer-based *in vitro* differentiation to illuminate functions of *dmrt5*. We corroborate our *in vitro* findings by global gene expression analysis and *in ovo* electroporation.

Results: Dmrt5 overexpression in ESCs and in the developing vertebrate neuroepithelium promotes the expression of ventral midbrain progenitor markers such as *Lmx1a*, *Foxa2* and *Msx1*. This is accompanied by concomitant inhibition of later marker gene expression. Global gene expression analysis indicates that *dmrt5* regulates a large number of genes expressed in the floor plate and basal plate of the midbrain. This indicates that it is capable of affecting patterning and cell fate choices in the midbrain. In contrast, loss-of-function studies in ESC show an inhibition of *Foxa2*, *Lmx1a* and *Msx1*, while lateral marker genes are unregulated. Finally, terminal differentiation of *dmrt5* overexpressing cell in minimal conditions shows an enhancement of dopaminergic neurons expressing key midbrain transcription factors.

Conclusions: Our study identifies Dmrt5 as a novel player involved in the complex regulation of ventral midbrain neuronal identities and dopamine neuron fate specification. It further demonstrates the power of ESCs as a valuable tool for illuminating gene function.

P47

Understanding the involvement of leucine-rich repeat kinase 2 in the endocytic-autophagic pathway

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Objective: To understand the involvement of leucine-rich repeat kinase 2 (LRRK2) in the endocytic-autophagic pathway using novel cellular models.

Background: LRRK2 is the most commonly mutated gene in familial Parkinson's disease (PD) and is also involved in sporadic disease. Understanding the function of LRRK2 and why it causes disease could therefore lead to the identification of new treatments.

We had previously found that in human brains LRRK2 localized to cytoplasmic puncta, the characterization of which would inform about the function of LRRK2. Autophagy is believed to be an important cellular mechanism preventing cell degeneration in long-lived cells. Since neurons survive for several decades, they are particularly sensitive to malfunction in the autophagy system.

Methods: In order to characterize LRRK2 puncta using a physiologically relevant model we used genomic DNA contained in a bacterial artificial chromosome (BAC) to express a YPet-LRRK2 fusion reporter in human cells. We then used immunoblotting, immunofluorescence and immunoelectron microscopy to characterize cells expressing wild-type and mutant LRRK2.

Results: We identified LRRK2 puncta as multivesicular bodies and autophagic vacuoles which belong to the endocytic-autophagic pathway. Interestingly, the R1441C pathogenic mutant showed a cellular phenotype of impaired autophagic balance. On the other hand, RNAi-induced knockdown of LRRK2 increased autophagic activity measured by LC3-II turnover.

Conclusions: Our data suggests that normal LRRK2 affects the activity of the autophagic pathway and that LRRK2 mutants could mediate disease by impairing the autophagic clearance balance. We are now developing primary neuronal cultures of BAC LRRK2 transgenic rats, as well as novel stable BAC LRRK2-expressing human neuronal cell lines, to allow for a more physiological study of the involvement of LRRK2 in autophagy pathways in neuronal models.

Investigating the dimerization and function of DJ-1 in living cells

P48

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Objective: In the present study we used bimolecular fluorescence complementation (BiFC) in living HEK-293T cells to gain insight into the function of DJ-1.

Background: Mutations in DJ-1 (PARK7), a small conserved protein of 189 amino acids, cause autosomal recessive PD (Bonifati et al, 2003). In the context of PD, DJ-1 appears to play a role in protecting cells from oxidative stress. Furthermore, crystallographic and biochemical studies have clearly shown that DJ-1 forms dimers, which likely play a critical role in its normal function (Wilson et al, 2003). However, the mechanism by which DJ-1 behaves as an "upstream" oxidative stress sensor in living cells, and the exact role of dimerisation in this process, is still unclear.

Methods: We fused one DJ-1 molecule to the N-terminal half of GFP (GN173), another DJ-1 molecule to the C-terminal half of GFP (GC155) and co-transfected both into cells. Fluorescence was analyzed via confocal microscopy, to ascertain whether DJ-1 dimers formed in living cells, and BiFC efficiency was evaluated via the Olympus Scan^R screening station.

Results: We clearly found that wild-type DJ-1 (wtDJ-1) is able to dimerize and is primarily localized to the cytoplasm in control conditions, whereas the L166P mutant of DJ-1 does not form dimers, in agreement with previous biochemical data. Moreover, L166P DJ-1 is not able to dimerize with wtDJ-1, as no green signal was observed when cells were transfected with the respective DJ-1 variants fused to the GFP halves.

Conclusions: Our results indicate that BiFC will serve as a robust and specific assay to study DJ-1 function in living cells. Future studies will elucidate the effects of the other two autosomal recessive DJ-1 mutations, M26I and E64D, on DJ-1 function and dimerization in normal and oxidative stress conditions.

P49

Modified dopamine signalling from neurons remaining after nigrostriatal dopamine lesion

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Objective: To explore activity dependence of dopamine (DA) release, and its control by cholinergic interneurons, in the striatum of mice with a partial dopaminergic nigrostriatal lesion.

Background: Loss of nigrostriatal DA neurons in Parkinson's disease (PD) may modify the function of the surviving DA neuronal network. Understanding this modification is important for developing strategies to sustain striatal DA function in PD.

Methods; Male C57BL6J mice (six to 10 weeks) received unilateral injections of 1 μ l 6-hydroxydopamine (6-OHDA; 4 μ g/ μ l in 0.9 % NaCl solution, 1% acetic acid). After two to three weeks acute brain slices were prepared for fast scan cyclic voltammetric recordings.

Results: Extracellular DA concentrations ([DA]_o) evoked by single electrical pulses in lesioned hemispheres were ~20% of those in contralateral hemispheres, with DA uptake also reduced. Application of stimulus trains revealed modified activity dependence of [DA]_o, and a reduction in the activity dependence usually produced by the nicotinic acetylcholine receptor (nAChRs) antagonist, dihydro- β -erythroidine (DH β E). These changes in regulation of [DA]_o were not necessarily due to altered nAChR function since cocaine, which inhibits DA uptake and increases Pr, attenuated the effect of DH β E in contralateral hemispheres. The relationship between extracellular calcium concentration and evoked [DA]_o was unchanged in lesioned hemispheres.

Conclusions: Together, these findings indicate a changed activity-dependence of DA signalling from neurons surviving a Parkinsonian lesion, due either to increased Pr via a mechanism that does not modify the calcium-dependence of release, or to modified DA uptake kinetics. This effect may compensate for the DA cell loss in PD. Furthermore, these data suggest that neuromodulatory drugs may influence dopamine transmission differently in the healthy versus Parkinsonian brain.

The role of Lmx1a and Lmx1b in midbrain dopamine neuron development

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Objective: Understanding the mechanisms regulated by Lmx1a and Lmx1b during differentiation of midbrain progenitors into mature dopaminergic neurons.

Background: Loss of midbrain dopaminergic neurons (mDA) represents the major cause of Parkinson's disease. Stem cells offer an opportunity to generate large numbers of standardized dopamine neurons for transplantation. However, incomplete re-innervation of dopamine neurons represents a major factor limiting transplantation success, underscoring the importance of identifying factors regulating mDA neurons axon projections. LIM-homeodomain transcription factors, Lmx1a and Lmx1b, are required for the development of midbrain dopaminergic neurons. However, the precise mechanisms regulated by these genes in midbrain dopaminergic cells remain to be discovered.

Methods: To study Lmx1a and Lmx1b function, we used mutant mice where these genes have been inactivated at different time points of mDA neuron development.

Results: Our results demonstrate that Lmx1a and Lmx1b play important and redundant roles in mDA progenitors, controlling their specification, proliferation and their differentiation. In addition, our preliminary data indicate that Lmx1a and Lmx1b regulate projection patterns of mDA neurons. Indeed, mutant mice for Lmx1a and Lmx1b failed to innervate their appropriate targets. We are currently investigating how Lmx1a and Lmx1b regulate mDA neuron connectivity.

Conclusions: Our data reveal that Lmx1a and Lmx1b play multiple roles during mDA neuron development. Altogether, our data will likely improve the differentiation of standardized midbrain dopaminergic neurons from stem cells thereby contributing to the efficiency of cell replacement therapies in Parkinson's disease.

P50

P51

Differentiation and characterisation of iPSCs generated from Parkinson's disease patients

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Objective: To generate iPSCs from fibroblasts from patients carrying genetic mutations in key genes involved in Parkinson's disease, and to differentiate these cells into neurons in order to study key aspects of dopamine homeostasis including content, synthesis, release and uptake. Further to this, we wish to modify key PD genes in iPSC cells lines using zinc finger nuclease technologies in order to generate cellular disease models.

Background: Induced pluripotent stem cells (iPSCs) present a tremendous opportunity for studying diseases as they can be prepared from accessible tissues and reprogrammed into virtually any cell type. Current studies of neurons from patients with neurodegenerative diseases are limited as they rely on data collected from post-mortem tissue. iPSC technology circumvents this and allows the study of cellular processes in early onset disease states and in subjects carrying disease-associated genetic mutations.

Methods: We have produced dopaminergic neurons from human embryonic stem cells (hESCs) and will now apply this protocol to patient-specific iPSC lines. HUES2 cells were directed towards dopaminergic neuronal differentiation by culturing in the presence of noggin, fibronectin and fibroblast growth factor. For terminal differentiation, growth factors were withdrawn and cells were grown in the presence of cAMP^(a).

Results: We have optimised a differentiation protocol in human embryonic stem cells that yields dopaminergic neurons efficiently. We will differentiate these cells into neuronal cells and then characterise their dopamine dynamics. In parallel, we will apply the same characterisation to iPSC lines after modification of PD genes using zinc finger technologies.

Conclusion: Together these approaches will greatly improve upon current research strategies within PD research.

^(a) Iacovitti, L *et al* (2007) Brain Research; 1127:19

Cell metabolism determines selective vulnerability in PINK1 associated Parkinson's disease

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Background: Mutations in PINK1 cause autosomal recessive Parkinson's disease (PD). PINK1 is ubiquitously expressed in all cells, but deficiency induces death selectively in neurons.

Objective: The aim of this study is to compare the effect of PINK1 deficiency on mitochondrial and cell metabolism in primary midbrain neurons and myotubes.

Methods: Live cell imaging and biochemical techniques were used to assess mitochondrial function and cell metabolism.

Results: We found that basal mitochondrial membrane potential ($\Delta\psi_m$) was decreased in PINK1 knockout (KO) neurons compared to wild type (WT) neurons. In contrast, basal $\Delta\psi_m$ was increased by $98.7 \pm 40.5\%$ in PINK1 KO skeletal myocytes compared to WT myocytes. Despite the difference in basal $\Delta\psi_m$, in both PINK1 KO neurons and myocytes, application of oligomycin induced mitochondrial depolarisation, suggesting that $\Delta\psi_m$ is partially maintained by the hydrolysis of ATP by F_1F_0 -ATPases, rather than solely by respiration. We believe this difference in basal $\Delta\psi_m$ is due to the higher glycolytic activity and ATP levels in myocytes compared to midbrain neurons. Furthermore, the high $\Delta\psi_m$ in myocytes protects cells against calcium induced mitochondrial depolarisation, which typically occurs in PINK1 deficient neurons. Prevention of calcium dysregulation in myocytes may result in sparing of these cells in Parkinson's disease.

Conclusions: Our results demonstrate that the same genetic defect (i.e. PINK1 KO) results in different pathophysiology in different cell types, depending upon the metabolic properties of the cell. This difference may contribute to neurons selective vulnerability in PINK1 associated PD.

P52

P53 Role of mitochondrial dynamics in neurodegeneration

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Mitochondria are continuously remodelled by fusion and fission events modulated by profusion proteins (Mfn1, Mfn2 and OPA1) and profission proteins (Drp1 and Fis1). The importance of the dynamic nature of mitochondria in the physiology of neurons has become clear from growing evidence showing association of common neuropathies and neurodegenerative diseases with defective mitochondrial dynamics. Recently, various studies have showed the direct and/or indirect interaction of disease associated proteins with the mitochondrial dynamics machinery. In particular, our group has recently reported that the PINK1/parkin pathway may regulate mitochondrial dynamics and mitophagy by targeting the pro-fusion protein Mfn.

We investigated the role of Mfn2 in neurodegeneration. To better understand the pathophysiological consequences of mutations in Mfn2, we studied fibroblasts from two Charcot-Marie-Tooth patients harboring mutations in the *Mfn2* gene.

Cells from patients with the most severe clinical phenotypes exhibited fragmented mitochondria with reduced branching and networks. Our results provide new insights into the role of pathogenic Mfn2 mutations in mitochondrial dynamics.

To further investigate the role of mitochondrial dynamics under physiological conditions we used *Drosophila melanogaster* as an in vivo animal model. *Drosophila* Mfn (also called Marf) shares 47% amino acid identity with both human Mfn1 and Mfn2.

Using an RNAi approach we down-regulated Mfn expression and found that Mfn ubiquitous knockdown mutants showed 3rd instar larval arrest, whereas nervous system Mfn knockdowns developed to adults but were poorly coordinated and showed reduced flight and climbing ability.

We also overexpressed Mfn using UAS-Mfn transgene and observed larval arrest in ubiquitous Mfn overexpression lines. Flies with Mfn overexpression in the nervous system reached adult hood but showed compromised flight and climbing abilities, also 80% showed abnormal wing posture. However, Mfn knockdown and overexpression only in motor neurons presented no major phenotypic defect.

Our results suggest that Mfn is playing an important yet unknown role during development and appeared critical for larval transition into pupal stage.

P54 Nedd4 ubiquitinates α -synuclein and promotes its degradation by lysosomes

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Objective: To understand how cells regulate α -synuclein, a critical protein for the onset of neurodegeneration.

Background: In Parkinson's disease, α -synuclein accumulates in intraneuronal inclusions often linked to ubiquitin chains, but the relevant degradative system and ubiquitin ligase regulating α -synuclein levels have been controversial. The proteasome, macroautophagy and chaperone-mediated autophagy have been reported to influence levels of overexpressed α -synuclein.

Results: The ubiquitin ligase, Nedd4, which functions in the endosomal-lysosomal pathway, ubiquitinates robustly α -synuclein, unlike other ligases previously implicated in this process. Nedd4 is expressed in the substantia nigra, in cells containing Lewy bodies and binds specifically to the carboxyl terminus of α -synuclein forming K63-linked ubiquitin chains. In the yeast α -synuclein model, disruption of the Nedd4 ortholog, Rsp5p, decreased α -synuclein degradation and enhanced inclusion formation and α -synuclein toxicity. In human dopaminergic neuroblastoma cells, Nedd4 overexpression enhanced endogenous α -synuclein clearance via lysosomes, while Nedd4 down-regulation increases α -synuclein content.

Conclusion: Ubiquitination by Nedd4 appears to target endogenous α -synuclein to the endosomal-lysosomal pathway, and by reducing α -synuclein content may help protect against the pathogenesis of Parkinson's disease and other α -synucleinopathies.

P55

Embryonic dopamine tissue grafts for Parkinson's disease. Are medical terminations a suitable source of tissue?

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Objective: Evaluation of tissue obtained from medical terminations of pregnancy for use in clinical trials for Parkinson's disease transplantation.

Background: TRANSEURO is an international project funded by the EU with the aim of re-initiating clinical trials of dopamine transplantation for the treatment of Parkinson's disease (PD). Due to the general trend in hospitals toward the use medical terminations of pregnancy (MTO) replacing surgical terminations of pregnancy (STOP) MTO will require detailed evaluation. An early goal of the TRANSEURO project is to investigate the suitability of dopaminergic tissue obtained from MTO for use in human transplantation.

Methods: Dopamine cell suspensions were prepared from ventral mesencephalic tissue obtained from MTO. They were evaluated in terms of cell numbers, viability, and dopamine cell yield, using in-vitro culture, and in-vivo transplantation into a rat model of PD.

Results: In the majority of embryos, the ventral mesencephalon contained healthy, viable cells capable of surviving dissection and disassociation into a cell suspension. Harvested tissue contained viable dopaminergic neurones producing up to 190,000 tyrosine hydroxylase positive cells per embryo in in-vitro cultures. Following transplantation into the rat brain, cell suspensions produced surviving grafts containing good numbers of tyrosine hydroxylase staining cells, which sent out axons into the surrounding host striatum.

Conclusions: So far, MTO tissue is comparable to STOP tissue on all of the indices used. Moreover, it looks to offer a more readily available source of tissue, allows more reliable dissection of midbrain dopamine neuron precursors, and looks a promising source of tissue for use in future human tissue trials.

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P56

Assessment of the presence and extent of vascular pathology and its influence on the clinical course of Parkinson's disease

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Objective: To investigate vascular risk factors to see whether they correlate with the presence of vascular pathology in the brain and affect the disease course of Parkinson's disease (PD).

Background: Many people affected by PD will have been exposed to vascular risk factors, which can affect blood vessel function in the brain, e.g. smoking, diabetes. It is well established that small blood vessel pathology contributes to the development of Alzheimer's disease (AD) in a subset of individuals and it has been proposed that vascular pathology may affect the disease course of PD.

Methods: We looked at 100 PD cases from the Parkinson's UK Brain Bank at Imperial College. The severity of PD and AD type pathology for each case was assessed with immunohistochemistry.

Magnetic Resonance Imaging (MRI) techniques were utilised to scan 30 cases with formalin fixed brain hemispheres to gauge the presence, size and location of white matter hyperintensities indicating damaged vasculature. Using MRI scans as a guide, areas of vascular pathology were sampled and tissue sections were stained with haematoxylin & eosin and luxol fast blue to confirm lesion location.

Tissue sections from MRI scanned cases and neuropathology blocks of all 100 cases were stained immunohistochemically with β -amyloid, ICAM-1, collagen-IV and smooth muscle actin to examine blood vessel wall integrity.

Finally the clinical histories were assessed to correlate the PD course, symptomology and cognitive decline with the presence of vascular pathology and vascular risk profiles.

Results: The majority of cases show some type of vascular pathology, only one of the MRI scanned cases showed no hyperintensities at all.

P57

Suspected Parkinson's: referral and first clinic visit to the specialist in the United Kingdom, 2009

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Objective: Our study aimed to assess the individual patient care for people referred to the clinic with the query: "Is this Parkinson's?"

Background: Parkinson's affects all aspects of daily life and impacts on individuals in different ways. The condition can be difficult to diagnose and challenging to manage. Although there are a range of drugs and therapies that deal with the symptoms, the successful treatment often depends on early diagnosis, differentiating Parkinson's from other similar conditions, and proper assessment of the condition.

Methods: 41 centres were asked to collect data from suspected Parkinson's patients referred to a geriatrician or neurologist for differential diagnosis. Data concerning assessment of the condition, differential diagnosis, access to Parkinson's drugs and other treatments as well as therapies and advice from a Parkinson's nurse was collected in the special Microsoft Excel spreadsheet retrospectively, using patient case notes. The data was collected for five months from 1 July to 30 November, 2009. 1,256 patients were included in the study, covering 60 NHS Trusts from England (covering all SHAs), Northern Ireland, Scotland, Wales and Guernsey Island.

Results: 37% of patients with suspected Parkinson's waited to see the specialist for differential diagnosis for more than six weeks with an average delay of 2.5 weeks. Six out of 41 centres saw all of their new patients within six weeks. 13.5% of suspected Parkinson's patients received drug treatment from a general practitioner or consultant before being referred for differential diagnosis. 15% of patients had their activities of daily living assessed. 24 participating centres were not using proforma when assessing activities of daily living. 56% of newly diagnosed patients had the documented assessment of speech and communication, and 43% had their swallowing assessed. 43% of new Parkinson's patients needed physiotherapy and 35% were referred for the therapy. Three out of 41 participating centres didn't have a Parkinson's disease nurse specialist service. One in every five patients was not offered Parkinson's disease nurse contact details, and just over a half of Parkinson's patients (57%) were offered written information about Parkinson's.

Conclusions: The study showed that there are a lot of gaps in differential diagnosis, assessment of the condition as well as therapy for suspected or newly diagnosed Parkinson's patients. Service differs in various geographical centres within the United Kingdom, which suggests existing inequalities in service for suspected or newly diagnosed Parkinson's cases.

P58

Drug treatment characteristics for people with Parkinson's in the United Kingdom

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Objectives: To investigate the prescription patterns and characteristics of drug treatment for Parkinson's patients within the United Kingdom.

Background: Parkinson's disease is a progressive neurological condition characterized by symptoms of tremor, rigidity and bradykinesia. Over 90% of patients receive drug therapy for their Parkinson's, and numerous clinical trials have been carried out to evaluate the efficacy of the most frequently used drugs. Our study investigated the prescription patterns and characteristics of Parkinson's patients using the largest sample survey of people with Parkinson's in the United Kingdom.

Methods: Self-reporting questionnaires were posted to 26,000 Parkinson's UK members in 2007 with a response rate of 50.6%. Twenty brand names of drugs were listed in the questionnaire asking participants to indicate their prescribed drugs. The ten most frequently taken drugs were the levodopa combinations Madopar, Sinimet and Stalevo, the catechol-o-methyl transferase inhibitor entacapone (Comtess), the monoamine oxidase B inhibitor rasagiline (Azilect) and dopamine agonists ropinirole (Requip), piramipexole (Mirapexin), pergolide (Carbaser), rotigotine (Neupro) and apomorphine hydrochloride (APO-go). These were then correlated with gender, age, demographic details, duration of illness and activities of daily life. From the total of 10,572 respondents with Parkinson's, 58.9% were males and 41.1% females. The mean age of respondents at the time of survey was 71.2 years and the average disease duration was 7.9 years.

Results: Madopar and Sinimet were the most commonly used drugs, and were used as monotherapy by one third of patients. Other drugs were more likely to be used in combination, with the mean number ranging from 2.1 to 3.4 drugs. Patients who took APO-go, Azilect, Mirapexin, Neupro and Requip for their Parkinson's were three to four years younger than the average, while Sinimet and Madopar users were found to be older than the mean 71 years old. APO-go, Carbaser and Comtess were taken by patients with the longest disease duration, with the median exceeding 10 years. Those patients who took Mirapexin, Requip and Azilect had a greater ability to carry out everyday activities, while those taking APO-go were least capable of carrying out such tasks.

Conclusions: Our surveys showed that levodopa, either as monotherapy or in combination with other drugs, dopamine agonists and monoamine oxidase B inhibitors, are the most frequently prescribed drugs for Parkinson's within United Kingdom.

P59

Should Parkinson's treatment be started immediately on diagnosis or delayed until functional disability develops?

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Objective: Meta-analyse randomised trials comparing early versus delayed start PD treatment.

Background: There is uncertainty whether treatment for PD should be started immediately on diagnosis or delayed until functional disability develops.

Methods: Data on change in UPDRS total score from baseline to final assessment were extracted for 1,777 patients in the three relevant trials identified and combined using standard meta-analysis methods.

Results: In two rasagiline trials, early treatment provided a 0.91 total UPDRS unit (95% CI: 0.01, 1.80) benefit compared with delayed treatment ($p=0.05$). There was apparent inconsistency in the trial results, with early treatment with 2mg significantly superior in the TEMPO trial and 1mg, but not 2mg, significantly better in the ADAGIO trial, making interpretation difficult. The PROUD study found no difference in UPDRS between immediate and delayed pramipexole (difference: -0.4; 95% CI: -2.2, 1.4; $p=0.65$). However, the results from the MAOBI and dopamine agonist delayed-start design trials did not differ significantly (test for interaction, $p=0.6$) and the combined results of the three trials showed a marginally significant advantage in UPDRS for early treatment (difference: -0.8; 95% CI: -1.6, -0.0; $p=0.05$).

Conclusions: Delayed-start trials suggest that PD patients may benefit from treatment being commenced immediately on diagnosis rather than when functional disability develops, with stronger evidence for MAOBI than dopamine agonists. Differences between 1mg and 2mg rasagiline were unanticipated and are likely chance findings. Whether there are worthwhile benefits in overall QoL from immediate treatment and the cost-effectiveness of this strategy remains uncertain.

Therefore, further large trials with long-term follow-up and a longer delay before starting treatment are needed, which include QoL outcome measures and health economics evaluation.

Biomechanics of Parkinson's disease while coming off Levodopa: a case study

P60

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Objective: This study aimed to describe biomechanically the changes in gait that occur as a patient with Parkinson's disease (PD) enters the off phase of the Levodopa (L-Dopa) cycle.

Background: Quantifying biomechanically the effect of L-Dopa on Parkinsonian gait has demonstrated that after administration a patient's speed and range of movements increase. However previous studies that examined the transition between 'on' and 'off' phase did not investigate the immediate changes that occur when the patient experiences entering the 'off' phase. This has significant implications for understanding the response to L-Dopa.

Methods: A single case study was conducted with a 60 year old male patient 10 years since diagnosis of PD. The participant performed a series of straight line walking tasks; initially while symptomatically in the 'on' phase and then 1.5hrs later when the effect was felt to have worn off. Kinematic data were collected using a ten camera Qualisys motion analysis system at 100 Hz. The segments of the lower limbs were modelled in six-degrees of freedom. The maximum, minimum and range of joint kinematics for the hip, knee and ankle were calculated.

Results: The overall speed of gait was found to be slower in the 'off' medication condition compared to the 'on' condition. Ankle plantarflexion during loading was not present in the 'off' condition and a significantly greater plantarflexion during propulsion was seen in the 'on' condition. Maximum knee flexion was significantly higher during stance phase and lower during swing phase in the 'off' condition. Hip flexion and extension were both significantly lower in the 'off' condition with the overall range of movement reduced by nearly a half.

Conclusions: This study demonstrates that as the short duration response to L-Dopa wears off the patient experiences an increase in symptoms and this is reflected by a significant change in the patient's gait.

P61

The Parkinson's Disease Everyday Cognition Questionnaire (PD-ECQ): A tool for measuring multi-domain everyday cognition in people with Parkinson's disease.

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Objective: To develop a questionnaire-based tool that measures the everyday manifestations of multi-domain cognitive impairments in early-stage Parkinson's disease (PD).

Background: Neuropsychological tests indicate that, even during early stages of PD, patients exhibit impairments in memory, attention and executive function. How these changes affect cognitive functioning in patients' everyday lives, however, is largely unknown. The PD Everyday Cognition Questionnaire (PD-ECQ) is being developed to address this issue of validity and clinical relevance.

Methods: Patients with PD (N=60) and healthy age-matched controls (N=33), screened for dementia and matched for years in education, completed a 136-item telephone questionnaire. Items measured everyday cognitive functioning, for example 'How often do you begin one task and get distracted into doing something else?'. Participants were asked to complete a battery of neuropsychological tasks, to assess validity.

Results: Thirty-six questions were selected from the larger questionnaire to form the PD-ECQ. Preliminary data suggest the tool successfully measures the everyday manifestations of cognitive dysfunction in early-stage PD. For example, self-reported planning ability was related to set-shifting errors ($p = .005$).

Conclusions: The PD-ECQ is able to capture everyday manifestations of the effects of PD on cognition. This potential assessment tool is currently being validated in a longitudinal study of the incidence of cognitive impairment in people with PD.

Glucocerebrosidase mutations in sporadic Parkinson's disease in North London

P62

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Objective: To determine the frequency of glucocerebrosidase mutations in sporadic PD in North London.

Methods: Direct sequencing of the entire glucocerebrosidase gene in 96 cases of PD from North London. Review of case records and clinical assessments to determine phenotype.

Results: 16/96 cases had a GBA sequence variant; seven were the polymorphism E326K, three were the polymorphism T369M, three N370S, one N188S, one RccNcII and one V460L. E326K and T369M are not considered as being risk alleles for PD (Sidransky et al. N Engl J Med 2009; 361:1651-61.) and the patients are not described herein.

Clinical information was available for three individuals with pathogenic changes. Patient 1 (N370S) a 50-year-old woman, presented with dragging of her left foot and a clumsy left hand. Her father and paternal uncle had PD. There was no tremor, bradykinesia of left arm and leg was noted. MRI brain was normal. Patient 2 (V460L) was a 75-year-old man. His main complaint was of postural instability. He was noted to have right-sided bradykinesia and rigidity with postural instability, but no tremor. Patient 3 (N370S) is a 67-year-old man who presented with tremor and freezing of gait. He had right sided tremor, bradykinesia and reduced right arm swing when walking.

Conclusion: 6.25% of PD patients in this North London cohort had a pathogenic GBA variant; this is in keeping with other studies. The phenotype of PD associated with GBA mutations in this series is very similar to that of sporadic PD.

P63

PD GEN: Parkinson's disease DNA Bank

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Objective: To develop a DNA bank from people with PD (PwPD) and controls using samples from large pragmatic randomised controlled trials (such as PD MED, PD REHAB and PD SURG) and to make these samples available to approved researchers working in the field.

Background: In the majority of cases, the causes of PD are unknown. There is some evidence for a genetic component or increased susceptibility to PD, but further work needs to be done to establish the genes involved and their role in the development of PD.

Methods: Participants in the PD MED, PD SURG and PD REHAB trials and their carers will be invited to give a small blood sample and answer an epidemiological questionnaire. The blood samples and questionnaires will be processed at the University of Birmingham. This will enable samples to be used based on specific pre-determined criteria eg PwPD who have smoked. Samples from carers will act as a control set of DNA. Anonymised DNA samples will be available to researchers on a case by case basis.

Results: To date (16 June 2010) the PD GEN DNA bank has 1259 samples: 807 from PwPD and 452 control samples from carers. The DNA bank is also starting to be used: it has provided the largest set of samples for the Wellcome funded Genome Wide Association Study (GWAS) for PD in the UK, which will be published shortly.

Conclusion: PD GEN is an important resource for research in to the genetic basis for PD. An understanding of the genes involved will lead to a significant step forward in our understanding of disease development.

Apomorphine use in the PD SURG trial

P64

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Background: As PD advances, many people experience less constant symptomatic relief from medications. At this point, there are a number of treatment options including deep brain stimulation (DBS), and continuous infusions of medications such as apomorphine and duodopa. The PD SURG trial compares the effects of DBS with best medical therapy which, in the UK, will often include apomorphine.

Aims: To compare apomorphine usage in the two arms of the PD SURG trial.

Methods: 366 participants were randomised to DBS or best medical therapy. Apomorphine usage, both dosage and route of administration, was recorded at baseline and at one year. Its changing usage by trial arm is reported below.

Results: Prior to entry into the trial and at baseline, there were no differences in the apomorphine usage by participants in each arm (Table 1), nor any difference in its planned use. However, by the one year time-point, there was a clear difference (Table 1): with more participants in the best medical therapy either continuing, or having started, to use apomorphine.

Conclusions: Apomorphine usage was considerably greater in the best medical therapy group. It is an expensive drug, hence, a large reduction in apomorphine use in the DBS arm is likely to be a significant factor in the planned cost-effectiveness analysis.

Table 1

Arm	Patients on apomorphine	
	DBS	Best medical therapy
Prior use*	72	73
Baseline	45	45
One year (x also at baseline)	13 (10)	63 (34)
Continuous infusion at 1 year	6	48
Intermittent doses at 1 year	7	15

*May still be on apomorphine at baseline.
2,328 characters (2,500 maximum)

P65 Systematic review of Levodopa dose equivalency reporting in PD

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Objective: To undertake a systematic review of studies reporting levodopa (LD) equivalency doses for anti-parkinsonian drugs and combine these data to provide standard formulae.

Background: Interpretation of clinical trials comparing different drug regimens for PD is complicated by the different dose intensities used: higher doses of LD and, possibly, other drugs produce better symptomatic control but more late complications. To address this problem, numerous conversion formulae have been reported for anti-Parkinsonian drugs that produce a total daily LD equivalent dose (LED) but no standard scheme has been adopted.

Methods: A systematic search to identify primary reports of LED estimates was performed. The LED of a drug was defined as that which produces the same anti-Parkinsonian effect as 100mg of immediate release LD (combined with a dopa decarboxylase inhibitor). Data on LED were extracted from each study and the mean and modal LEDs calculated. Where data were not available, LEDs were determined from information in manufacturer's reports and/or meta-analyses of clinical trials.

Results: 558 articles were identified from the initial searches, from which 56 primary reports with LED conversion formulae were included. Data from these studies yielded a standardized LED for each anti-Parkinsonian drug. For COMT inhibitors the LED is calculated by multiplying immediate release LD by 0.33 for entacapone, or 0.5 for tolcapone and adding to the total LD dose.

Conclusions: The standardized total daily LEDs described here provide a useful tool to express dose intensity of different anti-Parkinsonian drug regimens on a single scale. Using these conversion formulae to report LEDs would assist the interpretation of clinical trials comparing different PD medications.

Non-steroidal anti-inflammatory drugs (NSAIDs) as disease modifying agents for PD: evidence from observational studies

P66

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Objectives:

- Do NSAIDs prevent the onset of PD?
- Are NSAIDs neuroprotective in PD?
- What are the adverse effects (AEs) of taking NSAIDs in PD?

Background: Neuroinflammation may play a key role in the neurodegeneration associated with PD. NSAIDs may be beneficial in the primary and secondary prevention of PD.

Methods: Electronic databases and trial registers were searched, complemented by hand searching of conference proceedings and citation searching up to August 2009. For the primary prevention review, primary prevention trials and observational studies of NSAIDs were sought. Participants were free of PD when exposure to NSAIDs was assessed. For the secondary prevention review, trials in patients with well-defined PD were sought. Data were abstracted from the papers and methodological quality was assessed independently by two reviewers. Data were combined where appropriate using the inverse variance method.

Results: For the primary prevention review, 11 observational studies met the inclusion criteria. Exposure to any NSAID, or aspirin had no effect on the risk of developing PD. Exposure to non-aspirin NSAIDs reduced the risk of developing PD by 16% (table 1). Ibuprofen in isolation was examined in three studies and was associated with a 25% reduction in risk (table 1). No studies met the inclusion criteria for the secondary prevention review, and there was a lack of information on AEs.

Conclusions: There is currently no evidence for the use of NSAIDs in the secondary prevention of PD. Non-aspirin NSAIDs, are associated with a reduced risk of developing PD though this may or may not be causal. Further evidence, ideally from RCTs are required to confirm or refute these findings.

Table 1: Summary of effect estimates

	Effect Estimate (95% CI)
Non-aspirin NSAID	0.84 (0.87 to 1.01)
Ibuprofen	0.75 (0.64 to 0.89)

P67 H-pylori eradication for PD

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Objectives:

- What is the prevalence of *H-pylori* in PD patients?
- Does treatment of *H-pylori* infection with antibiotics improve symptoms in PD patients?

Background: LD is the main treatment for alleviating motor symptoms associated with PD. However, with time patients often experience fluctuations in symptoms and "wearing off" which may be related to variable absorption of LD. There is some evidence that treatment of the common gastrointestinal infection *H-Pylori* with antibiotics may improve LD absorption and hence improve symptoms.

Methods: Electronic databases and trial registers were searched, complemented by hand searching of conference proceedings and citation searching up to August 2009. Studies in patients with well-defined PD, who were *H-Pylori* positive, were sought. Data were abstracted from the papers and methodological quality was assessed independently by two reviewers. Recruitment figures for RCTs and other studies identified from the searching were used to determine the prevalence of *H-Pylori* in PD.

Results: Two completed and one ongoing trial met the inclusion criteria. One trial examined the effects of *H-Pylori* eradication on LD absorption and motor symptoms and found significant improvements in both. The other trial sought to find a causal link between infection with *H-Pylori* and Parkinsonism and was non-contributory. This trial reported a worsening of PD symptoms with *H-Pylori* eradication failure. The prevalence of *H-Pylori* in PD was reported in four studies and ranged from 37-59%.

Discussion: There is currently a lack of evidence for screening and treatment of *H-Pylori* in patients with PD. There is limited evidence to suggest that *H-Pylori* eradication improves the absorption of LD, and improves motor symptoms. Thus there remains a need for a well-conducted RCT.

Anti-hypertensive drugs (AHDs) as disease-modifying agents for Parkinson's: evidence from observational studies and clinical trials**P68**

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Objectives:

- Do AHDs prevent the onset of PD?
- Are AHDs disease modifying agents in PD?
- What are the adverse effects (AEs) of taking AHDs in patients with PD?

Background: Treatment for PD focuses on relieving symptoms. Potential neuroprotective or disease modifying agents have been identified in preclinical studies. One such group are AHDs.

Methods: Electronic databases and trial registers were searched, complemented by hand searching of conference proceedings and citation searching up to August 2009. For the primary prevention review, primary prevention trials and observational studies were sought. Participants were free of PD when exposure to AHDs was assessed. For the secondary prevention review, trials in patients with well-defined PD were sought. Data were abstracted by two reviewers.

Results: Three case-control studies met the inclusion criteria for the primary prevention review. The effects of calcium channel blockers and β -blockers on the risk of developing PD was examined in 2 studies, but the periods of exposure prior to PD onset were different, so results were not comparable.

Three trials and one ongoing trial (results due in 2012) met the inclusion criteria for the secondary prevention review. Each completed trial examined a different class of AHD (calcium channel blocker, ACE inhibitor and $\alpha 2$ adrenergic receptor agonist). AEs were noted in all three completed trials and included intolerability to the drugs and worsening PD symptoms.

Discussion: There is currently a lack of evidence for use of AHDs for either the primary or secondary prevention of PD. More observational studies are required to identify potential drugs to go forward for safety/tolerability studies in early PD.

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A large randomised assessment of the relative cost-effectiveness of different classes of drugs for PD (PD MED Trial)

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Objective: To describe the baseline characteristics of participants enrolled in the PD MED trial.

Background: PD MED is a large, simple, 'real-life' trial that aims to determine more reliably which class of drugs provides the most effective control, with the fewest side-effects, for both early and later PD.

Methods: Participants with early PD (previously untreated or with <6 months treatment) were randomised between DA, MAOBI and LD alone. Those with later PD (participants with motor complications uncontrolled by LD) were randomised between COMTI, DA and MAOBI. The main outcome measure is participant-rated quality of life (QoL), using the PDQ-39.

Results: PD MED closed to recruitment at the end of December 2009 with 1620 participants randomised in the early disease, and 500 in the later disease comparison. Participant age and gender were similar at baseline (Table 1). However, by definition, PD duration differed between the two randomisations. There was also a difference in H&Y stage, with most participants in the early disease randomisation in H&Y stage 1-2 compared to stage ≥ 2.5 for later disease participants. Most participants (92%) entering the early disease randomisation were PD treatment naïve. For participants entering the later disease randomisation, most (60%) were on LD alone when they developed motor complications. Participant QoL at baseline was poorer in participants in the later disease randomisation.

Conclusion: PD MED is the largest trial of medical therapy, and will provide unique evidence on the relative benefits of each class of drugs for the treatment of both early and later PD. The first results of the randomised comparisons will be available in 2011.

Table 1: Baseline characteristics of participants randomised into PD MED

		Early Disease (N = 1620)	Later Disease (N = 500)
Age (years)	Mean (SD)	70 (8.8)	73 (6.2)
Gender	Male	1053 (65%)	314 (63%)
Duration of PD (years)	Mean (SD)	0.6 (1.1)	5.6 (3.9)
Hoehn & Yahr Stage	1.0 – 1.5	815 (50%)	57 (11%)
	2.0	464 (29%)	147 (29%)
	2.5	234 (14%)	102 (20%)
	3.0	102 (6%)	154 (31%)
	4.0 – 5.0	5 (1%)	40 (9%)
PDQ-39: Mobility	Mean (SD)	29.4 (25.8)	49.2 (29.0)
PDQ-39: Summary Index	Mean (SD)	21.7 (13.4)	30.1 (15.2)

Synaptic plasticity induced by high- and low-frequency stimulation of cortico-subthalamic synapses

P70

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Objective: To determine the plasticity profile of glutamatergic (cortico-subthalamic) synapses in the subthalamic nucleus (STN) of control (dopamine intact) and dopamine-depleted animals.

Background: In Parkinson's disease (PD), neurones of the STN are hyperactive and display abnormal 'bursting' patterns of synchronous oscillatory activity, particularly in the beta (~20Hz) frequency range. Abnormal beta oscillations are associated with bradykinesia and reduced by dopamine replacement therapy or deep brain stimulation (DBS, 130Hz) of the STN. In animal models of PD, oscillatory activity of the STN correlates with enhanced cortical drive and this may be reduced by high frequency stimulation (HFS) of the motor cortex. Here, we show that it is only HFS in the dopamine-depleted state that produces long-term depression (LTD) of the 'hyperdirect' cortico-subthalamic pathway.

Methods: Whole-cell recordings were made from STN cells in parasagittal brain slices obtained from 200g dopamine-intact (in apomorphine 20 μ M) and 6-hydroxydopamine lesioned rats. EPSCs were recorded in the presence of GABAA receptor antagonist picrotoxin (20 μ M). In order to mimic DBS, HFS (100Hz for 1 second) was applied, while low-frequency stimulation (LFS: 10 stimuli at 40Hz every second for 5 minutes) was used to mimic the pathological firing patterns of STN neurones in lesioned animals.

Results: In dopamine-intact slices, HFS and LFS both produced long-term potentiation (LTP) (HFS, 133.3 \pm 4.3%, 4/9 slices; LFS, 148.2 \pm 24.7%, 6/8 slices). In contrast, in dopamine-depleted slices, HFS induced LTD (64.9 \pm 9.2%, 4/4 slices) while LFS only produced LTP in 2/6 slices (149.1 \pm 6.1%). Non-stationary fluctuation analysis, changes in paired-pulse ratio and frequency of spontaneous EPSCs indicate that LTP and LTD both have a presynaptic component associated with altered probability of glutamate release.

Conclusion: These results suggest that the beneficial effects of HFS in the dopamine-depleted state are partly due to the depression of cortico-subthalamic synapses. HFS and LFS stimulation paradigms in dopamine-intact and LFS in the dopamine-depleted state are predicted to have adverse effects on motor function.

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Specialist multidisciplinary rehabilitation for people with Parkinson's in the community: protocol of a RCT

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Objective: To present the protocol for a trial of specialist multidisciplinary rehabilitation for people with Parkinson's (PwP) in the community.

Background: A multidisciplinary approach to rehabilitation has not widely researched¹. In a previous study conducted by the research team, specialist rehabilitation was delivered to PwP and their carers in a day-hospital setting. Immediate benefits were not maintained when the treatment ended, and transportation for participants proved difficult and costly².

Methods: A subsequent trial is underway to investigate the cost-effectiveness of domiciliary rehabilitation, and the potential role of trained care assistants within a multidisciplinary team.

Results: A single-blind, randomised, controlled, 3-parallel arm, repeated measures, pragmatic design is being implemented; 270 PwP (at all disease stages) and their family carers are being recruited and randomised to one of three groups: Group A receiving six weeks of specialist multidisciplinary rehabilitation, and Group B receiving six weeks of rehabilitation and 18 weeks of care assistant support, and Group C (control) receiving usual care. Participants will be assessed at baseline (prior to randomisation), 6, 24 and 36 weeks by an independent assessor using a range of outcome measures including mobility, independence in ADL, health related QOL, carer strain. Acceptability of the interventions will be assessed through semi-structured interviews of key stakeholders. The trial incorporates an embedded economic evaluation.

Conclusions: The findings will inform future service development and improve quality of care for PwP and their carers.

- ¹ Gladman J et al (2007) *Specialist rehabilitation for neurological conditions*. Report for the NHS SDO R&D programme.
- ² Gage H et al (2006) *Clinical Rehabilitation*; 20:232-238.

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Content and use of individual home-based exercise and strategy programmes for repeat fallers with Parkinson's

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Objective: To describe the content and use of a menu of exercises and strategies in a randomised controlled trial (RCT).

Background: The effect of exercises and strategies for repeat fallers with Parkinson's was tested in a RCT previously reported¹. A menu of disease-specific and general exercises and strategies, with six levels of progression was developed for this trial.

Method: Independently mobile, cognitively intact people with Parkinson's were given personalised home-exercises by a physiotherapist for six weeks during an RCT of fall prevention. The exercises included strengthening, balance training, range of movement, walking and strategies for movement and safety. The therapist kept weekly records of prescription and progression. Subjects kept a record of participation. Progress was by change to a higher level of exercise complexity within the six levels; i.e. demand for greater movement coordination and balance, increased number of repetitions and increase in 'weights' used.

Result: Seventy participants were randomised to the exercise group, mean age 72 years and eight years from diagnosis, median Hoehn and Yahr rating 3. Individuals participated in a programme of personalised exercises, selected from the menu to address their specific needs. 74% received a unique combination of exercises. 86% of participants progressed to clinically judged higher level exercises during the treatment programme. Comparison between records of exercise prescription (therapist) and participation (people with Parkinson's), showed reduced uptake by people with Parkinson's in most cases.

Conclusion: This study illustrates how a structured progressive menu was used to develop individually tailored exercise and strategy programmes, demonstrate change in the exercise level over time and communicate the content of intervention used in the RCT.

- ¹ Ashburn A, et al (2007) 'A randomised controlled trial of a home-based exercise programme to reduce the risk of falling among people with Parkinson's disease': *JNNP*; 78: 678-684

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Promoting independent transfers: a feasibility trial of physiotherapy for Parkinson's disease (Pit:SToPP)

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Objective: To test the feasibility, in preparation for a large randomized controlled trial (RCT), of evaluating the effect of physiotherapy for improving transfers from sitting to standing of PwPD.

Background: 70% of people with Parkinson's disease (PwPD) have difficulty with transfers from different surfaces such as bed to chair; eight percent of falls among PwPD are associated with transfers.

Method: We recruited participants from Parkinson's UK local groups and hospital outpatient clinics; they had a confirmed diagnosis, were independently mobile, lived in the community and needed physiotherapy for transfers. The intervention (exercises and strategies) was delivered by a physiotherapist. Outcome was measured by a blinded assessor using video-recorded Sit to Stand time, PAS, Self-assessment PD Disability Scale, functional reach (FR) and the SS180 (turning test).

Results: 47 PwPD (35 male) were recruited, mean age 73 (SD6), range 58-86; median Hoehn & Yahr 3; 24 to the intervention group and 23 controls. We noted trends to improvement on the PAS, sit to stand, turn time and FR. The controls did not make the same level of improvement nor did they maintain it. Data from the posture item of UPDRS and quality of life measure were not distinctive. Feedback from 18/20 reported the intervention was helpful but 8/20 said the amount was insufficient. Nearly half the controls 7/17 were disappointed not to have physiotherapy.

Conclusion: Our intervention was feasible. Unexpectedly, for a small pilot, the intervention group improved over time, while the controls deteriorated. We recommend Sit to Stand, SS180, Functional Reach and Self Assessment Scale for outcome measures. Our findings will inform a larger study.

Improvements in the maximal voluntary force with loud auditory stimulation mimic the phenomenon of paradoxical kinesis in patients with Parkinson's disease

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Objective: To reproduce the phenomenon of Paradoxical Kinesis in a controlled experimental set-up by delivery of a loud auditory stimulus.

Background: Paradoxical kinesis describes the brief normalization of motor activity observed under circumstances of marked arousal in patients with Parkinson's disease (PD).

Method: Nine PD patients (mean age 61yrs, eight males) were instructed to grip a force dynamometer as quickly and strongly as possible in response to a VISUAL (V) cue (LED, 3s duration) delivered 20 times for each hand. A 0.3s duration, 1kHz, 106dB auditory stimulus was delivered at the same time as the V cue in ~50% of randomly selected trials (AUDITORY-VISUAL (AV) trials). The experiment was conducted after overnight withdrawal of anti-Parkinsonian medication, and again one hour after patients had taken their usual medication.

Results: General Linear Model (GLM) with factors L-DOPA (OFF & ON) and CUE (V & AV) for mean peak force identified a main effect of CUE ($F_{[1.00, 17.00]}=8.60$, $p=0.009$) independent of drug state (L-DOPA x CUE interaction ($F_{[1.00, 17.00]}=0.441$, $p=0.516$)), and no effect of L-DOPA. ($F_{[1.00, 17.00]}=0.11$, $p=0.917$). A GLM of mean peak rate of force development again showed an effect of CUE ($F_{[1.00, 17.00]}=08.22$, $p=0.011$), but no L-DOPA x CUE interaction ($F_{[1.00, 17.00]}=0.023$, $p=0.882$) nor effect of L-DOPA ($F_{[1.00, 17.00]}=2.64$, $p=0.123$). Averaging across drug states, mean increases in peak force and rate of force development of $7.6\pm 2.5\%$ (SEM) and $26.8\pm 6.9\%$ respectively were observed.

Conclusion: Both peak force and rate of force development increased with loud auditory stimulation. The relative independence of the mediating pathways from the dopaminergic system provides impetus for further investigation, as it may yield a novel non-dopaminergic target for therapeutic stimulation.

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Feasibility of abdominal massage for the alleviation of symptoms of constipation in people with Parkinson's disease

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Objectives: To determine the practicality and effectiveness of abdominal massage as an intervention for people with Parkinson's disease (PD) and constipation.

Background: Constipation is common in PD: prevalence estimates vary from 30–80%. Causes are multi-factorial but research suggests symptom severity is negatively correlated with quality of life; moreover, lack of research in this area means that bowel management must remain empirical (Coggrave et al, 2006).

Research has shown that abdominal massage can decrease symptom severity and have positive psychological effects (e.g. Lamas et al, 2009).

Method: Recruitment commences July 2010 and participants are eligible if they have PD, constipation as determined by the Rome III criteria and capacity to consent.

Outcome measures taken at:

- baseline (week 0)
- immediately post intervention (week six)
- four weeks post intervention

Measures include the Gastrointestinal Rating Scale, Constipation Scoring System, Neurogenic Bowel Dysfunction Score, International Consultation on Incontinence Modular Questionnaire and a Bowel Diary.

This is a prospective two-group single blind randomized controlled feasibility study (N=30).

Intervention phase (six weeks):

- both groups - weekly visits from clinician trained in abdominal massage
 - Group one (n=15) - bowel management advice and training in abdominal massage (patients and/or carers).
 - Group two (n=15) - bowel management advice
- Group one will be invited to participate in qualitative interviews.

Analysis: Data will be anonymised, entered blind onto a study database and analysed using SPSS.

Discussion: We hope this study will demonstrate the feasibility of this as an intervention in PD, and add to the existing evidence on the effectiveness of abdominal massage for constipation

High-frequency stimulation (HFS) of the subthalamic nucleus (STN) inhibits the firing of juxtacellular labelled 5-HT-containing neurons and decreases 5-HT release in vivo

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Objective: We studied whether prolonged STN HFS inhibits neurons in the dorsal raphe nucleus (DRN), whether responding neurons contain 5-HT, and whether 5-HT release decreases in the forebrain.

Background: STN HFS is an established surgical therapy in advanced PD. Despite beneficial motor effects this procedure often causes psychiatric side-effects including depression. Depression is strongly linked to decreases in 5-HT neurotransmission, and previously we reported that STN HFS for two minutes intervals inhibited the firing of putative 5-HT neurons in animal models.

Methods: Single unit recordings were made in the DRN of anaesthetised rats with neurobiotin filled glass microelectrodes. After baseline recording, the STN was stimulated (130 Hz, 100–200 μ A) bilaterally (5 or 20 min), after which neurons were juxtacellular-labelled and processed for neurobiotin-5-HT fluorescence immunocytochemistry. In microdialysis experiments extracellular 5-HT was monitored in the striatum and frontal cortex before, during and after bilateral STN stimulation (130 Hz, 100–200 μ A).

Results: STN stimulation inhibited (25–35%) DRN neuron firing, and this effect persisted over stimulation intervals of 20 minutes. Moreover, inhibited DRN neurons were 5-HT immunopositive. In microdialysis experiments, STN stimulation decreased extracellular 5-HT (20–30%) in both frontal cortex and striatum.

Conclusions: These data show that STN HFS decreases both the firing of 5-HT neurons as well as 5-HT release in the forebrain. Although the mechanism is not yet known, such changes may contribute to the psychiatric side-effects of STN stimulation in some PD patients.

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Effects of a yoga programme on an individual with Parkinson's disease: a single-subject design

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Objective: To investigate the effects of eight weekly yoga sessions on impairment, activity and participation in an individual with Parkinson's disease (PD).

Background: Yoga has properties that are thought to promote general fitness, strength, balance and general well-being and could be a valuable tool in the rehabilitation and treatment of people with PD. To date, only personal accounts have been made with regard to the benefits of yoga for people with PD.

Methods: A 69-year-old female with an eight-year history of PD (Hoehn and Yahr score two) was recruited for the study. A one-week baseline was followed by an eight-week period of weekly sixty minute yoga classes and a further five weeks of treatment withdrawal. Outcome measures consisted of the Berg Balance Scale (BBS), Timed Up and Go test (TUG) and the Parkinson's Disease Questionnaire-39 (PDQ-39). Outcome measures were collected at baseline (twice), midway during and at the end of intervention and at follow-up.

Results: No adverse effects were reported. An improvement was noted in the BBS (from 51 [out of 56] points at baseline to 54 at the end of intervention and 53 at follow-up) and TUG (from 13.2 sec at baseline to 12 sec at the end of intervention and at follow-up). No change in quality of life as measured by the PDQ-39 was noted.

Conclusions: We noted improvements in aspects of balance and mobility in our participant however, the improvements did not appear to be clinically significant. Subjectively, our participant gained much enjoyment and relaxation from the yoga classes. We believe this study justifies the need for further studies using a larger sample size.

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The effectiveness of different cueing devices for people with Parkinson's and gait initiation difficulties

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Objective: To investigate the effects of three cueing devices (visual, auditory, somatosensory) on movement and muscular control during gait initiation in people with Parkinson's disease (PD), who experience freezing.

Background: Sensory cues may help overcome difficulties in initiation of movement (freezing). Commercially available portable cueing devices claim to do this; it is currently unknown how effective these are.

Methods: Twenty participants with PD and freezing initiated walking under five randomised conditions: laser cane, sound metronome, vibrating metronome, walking stick and uncued. Data were collected using a 10 camera motion analysis system, force platforms and Electromyography. From 12 participants who exhibited freezing during testing, 91 freezing and 86 non-freezing trials were recorded.

Step length, centre of mass velocity (vCoM), centre of pressure velocity (vCoP) and number of zero crossings in the AP and ML directions were compared by independent T-test to determine the differences between freezing and non-freezing trials. One-way ANOVA tests with post-hoc pair-wise comparisons were used to test for differences between the conditions.

Results: Significant differences were seen in step length, vCoM, vCoP and number of zero crossings in the AP and ML directions between freezing and non-freezing episodes. Post hoc pair-wise comparisons showed a significantly greater vCoM and vCoP for the laser cane and walking stick, with significantly fewer zero crossings and greater step length for the laser cane.

Conclusions: This study has identified the laser cane as the most effective cueing device for people with PD and gait initiation difficulties over the other interventions tested in the gait laboratory. However the effectiveness and acceptability of such devices in use at home and outdoors has yet to be determined.

P79

Defining UK physiotherapy in Parkinson's disease: a modified Delphi survey

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Objective: To explore best practice physiotherapy for people with Parkinson's disease (PD) as perceived by therapists, and to obtain information on the current structure and delivery of physiotherapy services in the UK.

Background: Physiotherapy is viewed as an essential component in the management of PD, advocated by both patients and professionals. However, our understanding of what physiotherapy entails for this population is limited.

Methods: A two round, modified Delphi survey was conducted to generate information on the reasons for providing physiotherapy, the core areas of physiotherapy, perceived effective treatment techniques, and the measurement of outcome. In addition, an optional closed-questionnaire was disseminated to gain information on the characteristics of responding therapists, and the current structure and delivery of physiotherapy in the UK. Members of the Delphi panel consisted of researchers and practicing physiotherapists with an interest/expertise in the management of people with PD, and were recruited through personal correspondence, postings on the interactive Chartered Society of Physiotherapy web page, and advertisement at conferences. The survey was conducted from Primary Care Clinical Sciences at the University of Birmingham and disseminated to members of the Delphi panel via email.

Results: From a database of 107 therapists, 76 (71%) responded to the first round of the Delphi survey and 61 (80%) to the second round of the Delphi survey. In addition, 70 therapists completed the optional questionnaire. Full results to follow.

Conclusions: The findings of this survey will be used to provide a framework for the physiotherapeutic intervention delivered within a multi-centre randomised controlled trial investigating the clinical and cost effectiveness of physiotherapy and occupational therapy for people with PD: PD REHAB, and to inform clinical practice.

Long-term Individual Fitness Enablement (LIFE) for Parkinson's disease: A feasibility study

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Objective: To investigate the feasibility and acceptability of an individualised, supported community exercise programme for people with Parkinson's disease (PD).

Background: Exercise is a potentially important component within the management of PD. Habitual levels of physical activity are often lower for people with PD than age-matched healthy adults. Packages to support individualised community-based physical activity have not been investigated.

Methods: The study was conducted as a phase II RCT with blinded assessment at baseline and post-intervention. Adults with mild to moderate idiopathic PD were recruited. Participants randomised to the intervention group collaborated with fitness instructors to design a three-month individualised, progressive exercise programme. The intervention was delivered in leisure centres, and physiotherapy support was provided throughout. The control group received standard care. The primary outcome measure was the Physical Activity Scale for the Elderly (PASE). Secondary outcome measures included accelerometer monitored physical activity, the 10-metre and two-minute walk tests, Fatigue Severity Scale, Parkinson's Disease Questionnaire-39 and falls.

Results: Thirty-nine participants were recruited. Twenty were randomly assigned to the intervention group (five female; mean age 63 years) and 19 to the control group (three female; mean age 65 years). Uptake of the intervention was good (87% of participants: N=34) and the gym was attended well (mean number of 15 visits). There were no significant changes in any of the outcome measures. No adverse events were reported.

Conclusions: The trial confirmed the feasibility and acceptability of an individualised, supported exercise intervention, delivered in the community, for people with PD. In order to confirm the effectiveness of this intervention, a phase III RCT is now required.

P80

P81

PD REHAB: randomised controlled trial to study the effectiveness and cost-effectiveness of physiotherapy and occupational therapy for people with PD

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Objective: To provide information on the effectiveness and cost-effectiveness of physiotherapy (PT) and occupational therapy (OT) for Parkinson's as currently practised in the NHS.

Background: PT and OT are increasingly available for people with PD: increasing from 27% to 52% and 17% to 32% respectively from 1997 to 2008 in England (Parkinson's UK membership survey). Therapists and patients strongly believe that these therapies are effective, but to date, there is little evidence to support this view. Few clinical trials have been conducted and the outcome measures chosen have often given little information on effects on quality of life.

Methods: 750 participants will be recruited from ~40 centres around the country. Participants must: have idiopathic PD; report limitations in ADL; have no clear indication for or against PT/OT; must not be demented; must not have had PT/OT within the last year.

On agreeing to enter the trial, participants (and their carers if they so chose) will give consent, complete baseline questionnaires, and be randomised between immediate OT/PT and no OT/PT. In the active arm, PT and OT initial assessments should take place within four weeks of randomisation, then an individually tailored program will be designed around the needs of the participant and local practice.

Results: To date (16 June 2010) 34 sites have full approval with a further 10 potential sites. 94 participants have been randomised into the trial by 19 sites. Recruitment will be complete by July 2012 and follow up by October 2013.

Conclusion: PD REHAB will provide important evidence on the effectiveness of PT and OT and their cost-effectiveness, allowing informed decisions on their future provision within the NHS.

Does Parkinson's affect non-verbal gestures accompanying speech?

P82

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Objective: To establish whether Parkinson's alters non-verbal gestures that accompany speech and whether this affects communication success.

Background: Arm movements accompany speech and are closely tied to vocal activity. Speakers employ iconic gestures to supplement speech intelligibility and aid word finding. Parkinson's impairs voluntary arm movements. Examination of whether Parkinson's impacts on non-conscious and iconic arm movements accompanying speech and whether changes impair communication has been neglected.

Methods: Five people with Parkinson's in an 'on' state and five matched people without Parkinson's explained how to carry out three everyday tasks and mimed nine gestures representing everyday actions. We used a Vicon 3D motion capture system to measure angular displacement, velocity and acceleration of the shoulder, elbow and wrist joints and subsequently to create stylised moving images based on the reflective markers. Ten viewers blind to group membership rated whether they felt the 'person' had a movement impairment; 28 others indicated what they believed the gestures represented.

Results: In the conversation tasks, people with PD were significantly more impaired on elbow angle variation and velocity and shoulder angle, velocity and acceleration variation. During iconic gestures they showed significantly reduced elbow, wrist and shoulder angle metrics and shoulder velocity variation. Viewers were able to separate images of people with and without Parkinson's ($p < 0.01$). More gestures of people without Parkinson's were correctly recognised ($p < 0.003$).

Conclusions: Present data suggest people with Parkinson's demonstrate altered angle ranges and velocities in conversational speech gestures and when consciously miming actions. Further research is required to confirm on larger numbers and more varied tasks and to investigate other arm movement parameters that may be associated with reduced speech gestures.

P83

Disability and quality of life in Parkinson's disease is strongly associated with behavioural disorders

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Objective: To assess the relationship between the PD-related behavioural complications of apathy and ICD, and the outcomes of disability and health-related quality of life (HRQoL).

Introduction: Behavioural disorders of reward and motivation in PD including the impulse control disorders (ICD) and apathy are increasingly recognised. However, the impact of these complications on disability and HRQoL have received very little attention.

Methods: Single phase cross-sectional study with 99 community-dwelling, non-demented participants with PD, divided into three groups: ICD; apathy; and neither behavioural complication ("control"). ICDs were diagnosed using DSM-IV criteria and Apathy diagnosed by scoring ≥ 14 on the apathy scale (Starkstein 1992). Disability was measured in two ways: Part II of the UPDRS (UPDRS-Activities of Daily Living scale), and the Schwab-England scale; HRQoL was measured using the Parkinson's Disease Questionnaire (PDQ-8). Several other variables were collected including age at onset of PD, illness duration, the depression component of HADS (Hospital Anxiety and Depression Scale), impulsivity (Barrett Impulsiveness Scale), MMSE and the UPDRS-motor score. Forced entry linear regression models were created using either disability or HrQOL as the dependent models.

Results: 56% of the variance in disability ($p < 0.001$) is accounted for by higher levels of apathy, later stage of disease and more cognitive impairment; HRQoL shows the opposite pattern, with 54% of the variance ($p < 0.001$) being accounted for by higher impulsivity, younger onset, higher levels of depression, working memory deficits and higher dopaminergic load.

Conclusions: Disability and HRQoL are significantly, yet differentially, affected in participants with behavioural complications. It is very important to assess for ICDs and apathy as these have marked implications on PD patients' functioning and well-being.

Pain in Parkinson's disease and its relationship to illness perceptions and mood

P84

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Objective: To evaluate the relationships between pain, illness perceptions and mood in patients with Parkinson's disease (PD).

Background: Pain is a common but under-recognised non-motor feature of PD, with a prevalence of 40–80%. Some research suggests primary abnormalities of sensory processing in patients with PD, indicating a possible contribution of neuropathic pain mechanisms.

Although illness perceptions are associated with health outcomes in a variety of chronic conditions, little is understood about this relationship in PD. We hypothesised that more negative perceptions of PD and higher levels of depression and anxiety would be associated with higher levels of pain. We examined these associations in patients with different pain syndromes in PD.

Methods: Forty-seven consecutive non-demented patients with idiopathic PD and pain were evaluated using multiple pain measures, including the Short Form-McGill Pain Questionnaire, Leeds Assessment of Neuropathic Symptoms and Signs Scale (LANSS), and Neuropathic Pain Scale. Mood was assessed using the Hospital Anxiety and Depression Scale, and illness perceptions with the Brief Illness Perception Questionnaire. Parkinsonism was assessed using the UPDRS-III and -IV.

Results: Increased levels of pain were associated with more negative perceptions of illness, an interaction that was found to be mediated by depression. Significant differences in illness perception, and higher levels of anxiety, were identified in patients with probable neuropathic (n=18) compared to probable non-neuropathic pain (n=29), as defined by the LANSS score.

Conclusions: These findings highlight the interaction between pain and psychological factors in patients with PD. Improved pain control would require better understanding of the underlying pathophysiological mechanisms of pain and mood disorders in PD.

P85

Is turning different for people with Parkinson's disease?

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Objective: Examine the ability to distinguish mild from moderate disease severity, freezers from non-freezers and fallers from non-fallers in terms of their turning.

Background: Turning is a fall-related task and places people at risk of instability; people with Parkinson's disease (PwPD) take more steps and are slower than healthy controls¹.

Methods: We recruited PwPD and healthy controls (HC) from local Parkinson's UK groups. Turning was assessed in standing using the 180° Standing Start (SS180) test¹. Subjects turned 180° in both preferred and unpreferred directions. Time to turn, number of steps and quality of turning were documented. We collected UPDRS (motor) scores.

Results: We recruited 41 PwPD and 19 HC. PwPD [17 females] had a mean (SD) age of 69 (eight) years, mean (SD) duration of PD of seven (four) years and mean (SD) motor UPDRS score of 17 (six) points. HC [13 females] had a mean (SD) age of 68 (nine) years. Nearly two-thirds of PwPD (71%) and HC (68%) preferred to turn to their right. We found significant positive relationships between number of steps, time to turn, quality of turn and UPDRS ratings and significant differences between PwPD and HC (PwPD take longer and more steps and are less stable than HC). Worse ratings were found for those with increased disease severity and for freezers. We found one significant difference between fallers and non-fallers; time of preferred turn ($P=0.021$).

Conclusion: We confirmed that SS180 can distinguish between HC and PwPD and between mild and moderate condition. Also we demonstrated the SS180 can distinguish between freezers and non-freezers. We did not demonstrate a distinction between repeat fallers and single or no fallers.

- ¹ Stack E, Ashburn A (2008) 'Dysfunctional Turning in Parkinson's disease' *Disability & Rehabilitation*; 30 (16): 1204

A community-based study of Parkinson's non-motor symptoms

P86

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Parkinson's UK

Objective: Non-motor symptoms are a key factor affecting the quality of life of people with Parkinson's. The aim of the current study was to investigate the prevalence of non-motor symptoms in a community-based setting using the NMS-Quest rating scale.

Background: There is an increasing awareness of the importance of non-motor symptoms for people with Parkinson's. A number of studies have been carried out into the prevalence of specific symptoms and to investigate their effects on the quality of life of people with Parkinson's. Such symptoms may also be associated with an increased need for medical, nursing and institutional care.

Methods: A survey containing the NMS-Quest non-motor scale was posted to 26,000 members of Parkinson's UK with 10,400 completed questionnaires being returned by people with Parkinson's. Other data that was obtained included gender, age, demographic details, duration of illness, quality of life (PDQ-8) and medication. The NMS-Quest scale assesses thirty symptoms grouped into ten domains.

Results: The prevalence of the individual symptoms varied among the patient population from bowel incontinence in 10% of the population to nocturia in 45%. There were also gender differences with men having greater problems with sex drive and difficulty, with women having a greater incidence of anxiety and depression. The prevalence increased with illness duration although this was independent of age. Furthermore, nocturia and loss of taste and smell predated the onset of motor symptoms.

Conclusions: The results from the current community-based study correlated well with the initial development and validity of the NMS-Quest scale which was clinically based. This confirms its usefulness as a tool of assessing the non-motor symptom load of people with Parkinson's. Furthermore, the self-assessment format allows for large numbers of people to be assessed in a non-clinical setting.

P87

Incidence of cognitive impairment in cohorts with longitudinal evaluation – Parkinson's disease (ICICLE-PD) study

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Objectives:

- 1 To accurately characterise two independent cohorts of incident Parkinson's disease using strict epidemiological criteria and a variety of assessments (clinical, biochemical, genetic and imaging).
- 2 Longitudinal follow-up of incident cohorts to identify risk factors and biomarkers for cognitive impairment and Parkinson's disease dementia (PDD).
- 3 Establish a simplified panel of tests to help predict PDD.
- 4 Provide further information on the pathophysiological processes that lead to PDD and avenues to develop further interventional therapy.

Background: Dementia occurs more frequently in association with Parkinson's disease (PD) compared to a normal age-matched control population. If followed for long enough, up to 80% of people with PD may develop dementia. ① This is of huge significance now, as our treatment of the motor features of PD with levodopa and other drugs is becoming more successful, thus giving dementia time to emerge.

Although the prospect of someone with PD becoming demented is upsetting for all concerned, it is clearly important to identify individuals at high risk of this complication, so that appropriate therapy and management decisions can be made. One of the main aims of the ICICLE-PD study is to better understand the transition between normal cognition and dementia in people with PD. We may then be able to predict the onset of dementia and stratify risk for patients with PD.

Methods:

- 1 Identification of two incident cohorts of PD via validated criteria (UK Brain Bank criteria) over a 30 month period (recruitment commenced on 1 June 2009).
- 2 Longitudinal clinical assessment at 0, 18 and 36 months after recruitment into study.
- 3 Assessments to include clinical, biochemical, imaging (structural and functional MRI as well as FDG-PET scans).

Current progress: A total of 135 participants have been recruited in both centres: Newcastle-upon-Tyne and Cambridge, for the clinical study. The current crude incidence rate of PD in the north east of England is 14.2/100,000/ year. Good progress is being made with the clinical assessments and data entry. The database will provide a rich resource for this and future studies.

Preliminary results: Preliminary results of the first 50 consecutive participants (mean age 67.3 years, range 35-84 years) who have completed both Mini Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA) reveal a mean score of 28.8 out of 30 and 25.8 out of 30, respectively. 20 (40.0%) participants had a MoCA score of ≤ 25 , the recommended cut-off point for cognitive impairment. 17 (34.0%) participants had a MMSE score of ≥ 26 and a MoCA score of ≤ 25 . 2 participants had a MMSE score of < 26 meeting one of the criteria for the diagnosis of PDD.

Conclusion: As per recent evidence ②, the MoCA may be more sensitive as a screening instrument for cognitive impairment in PD when compared to the MMSE. Cognitive impairment in recently diagnosed PD is likely to be higher than previously reported. Suitable and in-depth cognitive tests are required in the routine clinical assessment in PD. More research is required in the field of cognitive impairment in PD and its prognostic value for predicting subsequent dementia. Further longitudinal data from the ICICLE-PD study will improve our knowledge in this area.

References:

- ① Hely Mariese A, Reid Wayne GJ, Adena Michael A, Halliday Glenda M and Morris John GL (2008) 'The Sydney Multicentre Study of Parkinson's Disease: The Inevitability of Dementia at 20 years' *Movement Disorders*, 23, pp. 837-844.
- ② Hoops S, Nazem S, Siderowf AD, Duda JE, Xie SX, Stern MB, and Weintraub D (2009) 'Validity of the MoCA and MMSE in the detection of MCI and dementia in Parkinson disease' *Neurology*, 73, pp. 1738-1745

P88

Evaluation of voice tremor in idiopathic Parkinson's disease (PD)

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Objective: To describe the nature and source of voice tremor in PD, identify ways in which it can be measured, and explore relationships between voice disability and PD disease variables.

Background: Speech and voice changes are highly prevalent in people with PD. The voice symptoms are salient. Voice tremor is frequently reported as a feature of the PD speech-voice symptom complex. However, little is known about its characteristics, ways to identify or measure it, or its relationship to voice disability and PD non-speech symptoms. Acoustic evaluation, which permits the quantification of specified voice features, has not been used specifically to measure the tremor component in the PD voice. It remains unclear how voice tremor might impact on communication and how it relates to non-speech changes associated with PD.

Methods: We report an ongoing study of 30 people with PD and age-sex matched controls. The study employs visual (laryngoscopic), perceptual and acoustic measurement approaches to identify and quantify voice tremor. In this presentation, the focus is on the preliminary acoustic findings, using measures of frequency variability, magnitude of frequency tremor, and amplitude tremor. Voice disability is assessed using the Voice Handicap Index (VHI) and PD motor symptoms rated using the Unified Parkinson's Disease Rating Scale (UPDRS).

Results: We will report results of preliminary acoustic voice tremor analyses of 30 people with PD, with analyses of associations of presence and severity of tremor with self-rated VHI and motor changes (UPDRS).

Conclusions: We anticipate that results will be able to address key issues in speech motor control, assessment and treatment in the speech-language therapy clinic. They will contribute to the debate on the nature, origins, measurement and relationships of tremor in PD.

Investigating co-speech gesture production in Parkinson's disease

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Objective: To determine whether Parkinsonian patients produce significantly fewer co-speech gestures than age-matched controls and if any gesture type is particularly affected.

Background: In healthy people, gestures alongside speech can add meaning and emphasis to speech. There is some evidence that gestures are reduced by Parkinson's disease (PD), alongside other motor problems. Conversely, gestures might provide a means to compensate for poor speech in PD. To date, there has been very little research on the effect of PD on gestural production and communication.

Methods: 23 Parkinsonian patients [Hoehn and Yahr stage III or less, mean (SD) motor score on UPDRS = 20.10 (6.60)] and 22 healthy controls took part. Video data was collected following an experimental session, while participants described what they had done. Each participant's speech was transcribed and their gestural movements identified. Each gesture was classified, using an established scheme, as iconic, metaphoric, deictic, interactive or a beat.

Results: There were no significant differences in numbers of gestures (per 100 words) between the two groups, either overall or when split up according to type. However, those iconic gestures used to describe actions were qualitatively different, namely less precise in the Parkinson's group.

Conclusions: Contrary to our expectations, gesture rate was not significantly affected in this group, with relatively mild PD. This may indicate that co-speech gestures could compensate for speech problems. However, the communicative value of those gestures representing actions was diminished. This study demonstrates the value and feasibility of carrying out fine-grained analysis of gestures in PD. The qualitative change should be investigated further and gesture production examined in a wider range of communicative situations.

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P90

Objective and clinically feasible assessment of spinal posture in people with Parkinson's disease

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Objective: To investigate spinal posture in people with Parkinson's disease (PwPD) and the relation between spinal posture and movement difficulties.

Background: Postural adaptations such as the stooped and forward, inclined position are usually reported for PwPD but the assessment of spinal posture has only been done by means of complex and laboratory equipment which are clinically unfeasible and not user-friendly.

Methods: PwPD and healthy controls (HC) from Parkinson's UK local groups were assessed with a small and simple hand-held device (SpinalMouse®) in sitting and standing, in the upright, flexed and extended position. Variables collected were thoracic and lumbar spinal curvature, and sacral and total spinal inclination. PD movement difficulties were collected by means of the UPDRS, part III.

Results: We recruited 37 PwPD (17 females, mean±SD age 69±8 years, mean duration of PD 6±4 years, mean motor UPDRS score 17±5 points) and 19 HC (13 females, mean age 68±9 years). PwPD had an increased thoracic kyphosis in upright sitting ($p<0.000$) and standing ($p=0.014$). For PwPD, the sacrum was significantly tilted more backwards in upright sitting ($p=0.006$), flexed sitting ($p=0.038$) and extended sitting ($p=0.013$). In standing, PwPD's total spinal inclination was significantly more anterior in the upright posture ($p<0.000$) and significantly less posterior when asked to lean backwards ($p=0.009$). Both latter results were significantly correlated with PD movement difficulties ($r=0.33-0.42$).

Conclusions: We demonstrated objectively that with a clinically feasible device such as the SpinalMouse®, PwPD had an altered spinal posture in comparison with HC which were related to movement difficulties.

These changes could become the focus of postural rehabilitation of PwPD, both in a research and clinical setting.

P91

Perceptions of cause and control of impulse control behaviours in people with Parkinson's disease

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Objectives: Impulse control behaviours (ICBs) have been a recent focus of research in people with Parkinson's disease (PD). However, the current literature is limited to a biomedical understanding and gaps remain in our understanding of the cause of these behaviours and how they are experienced by the people themselves. Consequently, this study sought to investigate how people with PD perceive the cause and controllability of their ICB.

Design: The study utilised qualitative methodology involving semi-structured interviews. Interpretative phenomenological analysis (IPA) allowed an in-depth exploration of the subjective experience of ICBs.

Methods: 10 people with idiopathic PD and current or recent history of ICBs were recruited from an existing research participant pool.

Results: The themes which arose from the participants' accounts were *'It does seem to open a whole Pandora's Box of who we are and why we do what we do; conflicting views on causality'; 'Better to live like a tiger for a day than like a lamb for a year; impulse control behaviours as a coping strategy.'*; and *'Just a thing I couldn't control, like a greater power than me; relationship between causal attribution and perceived controllability.'*

Conclusion: Participants' beliefs about the cause of ICBs varied from externalised cause (medication) to internalised (coping with the impact of PD). These causal attributions were fundamental to the perceived controllability of the behaviours and psychological benefits. Further research is warranted to explore a psychosocial viewpoint of this feature of PD and to provide appropriate and effective biopsychosocial interventions.

P92

Neuroprotective effect of GLP-1 receptor agonist Exendin-4 in 6-OHDA rat model involves D₃ receptor mediated changes in neural precursor cells

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Objective: To demonstrate that the effect of Exendin-4 (EX-4) in the 6-OHDA model of PD is D₃ receptor dependent and measure the effect on cells in the subventricular zone (SVZ).

Background: EX-4 is protective in models of PD (Harkavyi *et al*, 2008) and promotes neurogenesis (Bertilsson *et al*, 2008), suggesting therapeutic value in neurodegenerative disorders.

Methods: Male Wistar rats were treated with 6-OHDA stereotaxically. A week later, rats were given EX-4 (0.5 µg kg⁻¹), 5-bromo-2-deoxyuridine (BrdU) (50mg kg⁻¹) and nafadotride (NAF; D₃ antagonist 1mg kg⁻¹) twice daily. Seven days later, rats were challenged with 0.5 mg kg⁻¹ of apomorphine to evaluate contralateral circling. They were then implanted with microdialysis probes and perfused with aCSF, samples collected and DA levels estimated using HPLC. Brains were then processed for tyrosine hydroxylase (TH) and BrdU immunohistochemistry (IHC).

Results: 6-OHDA-only treated rats circled intensely (23 ± 3 turn/120 s) with apomorphine. This was attenuated by EX-4 (3 ± 1 turns/120s) and reversed by NAF (17 ± 2 turns/120s). Extracellular DA was also reduced with NAF. TH IHC showed reduced TH+ cells in EX-4 + NAF groups compared to EX-4 only in which TH staining was similar to controls. BrdU IHC in SVZ revealed reduced numbers of BrdU+ cells in EX-4 treated groups and was increased in groups given NAF.

Conclusion: Results show that EX-4 restores dopaminergic function and reduces the numbers of BrdU-positive cells in the SVZ. Both phenomena appear to be D₃-receptor dependent. This possibly means that EX-4 promotes cell migration from the SVZ.

Neuroprotective effects of FGF-20 on ventral mesencephalic embryonic dopamine neurons and in the partial 6-OHDA rat model of Parkinson's disease

P93

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Objective: This study aimed to confirm FGF-20's reported neuroprotective effects in ventral mesencephalic (VM) embryonic dopamine neurons, and to evaluate if FGF-20 has neuroprotective effects in the partial 6-OHDA rat model of PD.

Background: FGF-20 and its receptors are present in the SNc and the striatum. *In vitro*, FGF-20 protects dopamine neurons against serum withdrawal, glutamate toxicity and 6-OHDA. These findings indicate that FGF-20 might have neuroprotective potential in PD.

Methods: VM cultures were treated with FGF-20 for 24 hours on DIV7. On DIV8, cultures were exposed to 6-OHDA for four hours. On DIV9, cultures were fixed and stained for TH. For the *in vivo* study, partial unilateral 6-OHDA nigrostriatal lesions were induced in rats. Chronic supra-nigral FGF-20 (1 µg and 2.5 µg/day) infusions were started one day prior to lesioning. Motor function was measured at intervals using the cylinder test. Nigrostriatal lesion size was quantified by TH immunohistochemistry.

Results: In the cell studies, 6-OHDA induced an ~60% reduction in TH+ cells vs vehicle, and FGF-20 significantly protected against this cell death. In the *in vivo* experiments, the vehicle-treated 6-OHDA lesioned rats had an ~65% lesion. In the rats receiving the 2.5 µg/day FGF-20 treatment, striatal TH levels and TH+ nigral cell counts were preserved at significantly higher levels vs vehicle. Motor function in the cylinder test was also preserved in the 2.5 µg/day group.

Conclusions: These results confirm FGF-20's ability to protect dopamine neurons *in vitro*. We also show for the first time that FGF-20's protective effects are also present *in vivo*, in the partial 6-OHDA rat model of PD. These findings provide further support for FGF-20's neuroprotective potential in PD.

P94

The expression of nicotinamide N-methyltransferase increases the toxicity of tryptoline in-vitro

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Objective: To determine whether the expression of nicotinamide N-methyltransferase (NNMT) enhances the toxicity of tryptoline towards SH-SY5Y neuroblastoma cells.

Background: NNMT is responsible for the N-methylation of nicotinamide to 1-methylnicotinamide. NNMT is significantly over-expressed in the brains of patients who have died of Parkinson's disease (PD), with an inverse correlation between NNMT expression and disease duration (Parsons et al (2002) *J Neuropathol Exp Neurol*; 61:111–124). The over-expression of NNMT in SH-SY5Y cells, which have no endogenous NNMT expression, protects SH-SY5Y cells from the toxicity of various mitochondrial toxins. The current study is designed to assess whether the expression of NNMT enhances the toxicity of tryptoline, produced endogenously from tryptophan and present in the PD brain, whose N-methylated derivatives are Complex I inhibitors.

Methods: the expression of NNMT in SH-SY5Y cells and SH-SY5Y stably expressing NNMT (named S.NNMT.LP) was confirmed using RT-PCR and Western blotting. The toxicity of tryptoline was compared in both cell-lines using LDH release. Free radical production was measured indirectly using GSH:GSSG ratio, and lipid peroxidation was measured using 8-isoprostane concentration.

Results: RT-PCR and Western blotting confirmed that parental SH-SY5Y cells expressed no endogenous NNMT, whereas S.NNMT.LP cells expressed robust levels of NNMT. Tryptoline was only significantly toxic towards SH-SY5Y cells at 0.5mM and greater, whereas toxicity was observed towards S.NNMT.LP cells at 0.125mM and greater in a dose-dependent manner, which was higher than that observed towards SH-SY5Y. Tryptoline decreased the GSH:GSSG ratio and increased lipid peroxidation in both cell-lines.

Conclusions: the expression of NNMT predisposes SH-SY5Y cells to tryptoline toxicity. This increase in toxicity may arise from increased free radical production, although this requires further investigation.

Pharmacological manipulation of peroxisome proliferator-activated receptor γ reveals a role for anti-oxidant protection in a model of Parkinson's

P95

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Objective: Exploration of the impact of the peroxisome proliferator-activated receptor γ (PPAR γ) activity on the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of Parkinson's disease, focusing on oxidative stress mechanisms.

Background: PPAR γ agonists have been shown to provide neuroprotection in a number of neurodegenerative diseases, including Parkinson's. These protective effects are primarily considered to result from the anti-inflammatory actions of PPAR γ , however, there is increasing evidence that anti-oxidant mechanisms may also contribute.

Methods: The effects of PPAR γ agonist rosiglitazone and the antagonist GW9662 *in vitro* in SH-SY5Y cells and *in vivo* in C57BL6 mice on MPP+/MPTP toxicity were assessed, using cytotoxicity assays, Western blotting, qPCR, immunohistochemistry, stereological counting and HPLC.

Results: Rosiglitazone attenuated reactive oxygen species formation induced by MPP+ in SH-SY5Y cells in a PPAR γ -independent way, involving upregulation of glutathione-S-transferase activity, but not superoxide dismutase activity. The localisation of PPAR γ *in vivo* to dopaminergic neurons of the substantia nigra pars compacta (SNpc) was established by immunohistochemistry and PPAR γ levels were found to be upregulated seven days after MPTP treatment. The importance of PPAR γ in protecting against MPTP toxicity was confirmed by treating C57BL6 mice with GW9662. Treatment with GW9662 increased MPTP-induced neuronal loss in the SNpc while not affecting reductions in striatal dopamine and 3,4-dihydroxyphenylacetic acid. GW9662 also caused neuronal loss in saline-treated mice, which correlated with reductions in glutathione-S-transferase pi mRNA levels.

Conclusions: The evidence presented here further supports the role of anti-oxidant mechanisms in the protective effects of PPAR γ agonists in neurodegenerative diseases. It demonstrates the importance of PPAR γ activity for neuronal survival.

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Regulation of dopamine release by discrete striatal nicotinic acetylcholine receptors in dorsal versus ventral striatum

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Objective: In this study we explored the identity of nAChRs that regulate dopamine transmission in caudate putamen (CPu) and in the nucleus accumbens (NAc).

Background: Most forms of therapy for Parkinson's disease (PD) lose efficacy with time and produce drug-induced side effects necessitating the need for alternatives. Nicotine acting at nicotinic acetylcholine receptors (nAChRs) can powerfully modulate (and enhance) striatal dopamine release. The heteromeric receptors that govern striatal dopamine release can be broadly divided into $\alpha 4\beta 2$ -containing (*), or $\alpha 6\beta 2^*$ and it remains unresolved whether these are distinct, or overlapping populations (i.e. $\alpha 4\alpha 6\beta 2$ -nAChRs) or involve other subunits, e.g. $\alpha 5$, $\alpha 3$, $\beta 3$. If nAChRs are to be considered for a potential PD therapy, it is imperative to characterise their specific subunit identify.

Methods: Action potential-dependent dopamine release was observed in real-time, using fast-scan cyclic voltammetry at carbon-fibre microelectrodes in striatal slices from $\alpha 6$ - or $\alpha 4$ -null mice.

Results: In CPu, deletion of either subunit had limited net impact on ACh-regulated dopamine release. However, subunit deletion did result in a compensatory up-regulation of the complementary α subunit, suggesting that distinct $\alpha 4^*$ and $\alpha 6^*$ -nAChRs are functional in CPu. In contrast in NAc, the loss of either subunit significantly attenuated ACh control of dopamine release, suggesting control by a combined $\alpha 4\alpha 6\beta 2^*$ -nAChR.

Conclusions: These data therefore identify distinct nAChRs that govern striatal dopamine release in CPu versus NAc, identifying two important targets for regulation of CPu dopamine: $\alpha 4(\text{non-}\alpha 6)\beta 2^*$ - and $\alpha 6\beta 2^*$ -nAChRs.

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Selective activation of the mGlu4 but not mGlu7 group III metabotropic glutamate receptor provides functional neuroprotection in an animal model of Parkinson's disease

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Objective: The aim of this study was to examine whether activation of either mGlu4 or mGlu7 group III mGlu receptors alone was sufficient to mediate functional neuroprotection in the 6-OHDA lesion model of Parkinson's disease (PD).

Background: Previously we identified the broad spectrum agonist L-AP4, that stimulates mGlu4, 7, and 8 receptors significantly preserves the nigrostriatal tract. Our objective here was to elucidate the key group III mGlu receptors underlying the observed neuroprotection ①.

Methods: Male Sprague Dawley rats were cannulated above the right SNc prior to unilateral 6-OHDA induced lesion of the nigrostriatal tract. Supranigral injections of the mGlu4 positive allosteric modulator, VU0155041 (10–100nmol), the selective mGlu7 allosteric agonist, AMN082 (1–100nmol) or respective vehicle were given 1h before and daily for 7 days after lesion. Motor function was assessed at intervals using the cylinder, adjusted steps tests and the amphetamine-induced rotational test to provide an index of nigrostriatal function. The extent of dopaminergic denervation was confirmed post-mortem using HPLC measurement of striatal dopamine content, and TH immunohistochemistry in the striatum and SN.

Results: Treatment with VU0155041 produced significant protection of the nigrostriatal tract with respect to dopamine levels in the striatum and against dopaminergic cell loss in the SNc, whilst further demonstrating functional improvement in the behavior of these animals. In stark contrast, AMN082, failed to mediate functional preservation of motor behaviour, nor offered protection at a neurochemical or immunohistochemical level.

Conclusion: These findings demonstrate that, of the group III mGlu receptors investigated to date, mGlu4 offers the most promising target for establishing disease modification in PD.

① Austin P et al (2010). *Br J Pharmacol* In Press.

P98

Characterisation of novel LRRK2 monoclonal antibodies for human tissue use

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Objective: To characterise new monoclonal antibodies for LRRK2, and assess their specificity on human post-mortem brain tissue.

Background: Mutations in the LRRK2 gene are the most common cause of late-onset autosomal-dominant Parkinson's disease (PD) and are also prevalent in sporadic PD cases. LRRK2 is a large multi-domain protein with putative enzymatic functions, however, its function in PD pathology remains unknown. LRRK2 is an active kinase *in vitro*, and mutations in the kinase and GTPase domains affect kinase activity. The G2019S mutation in the kinase domain promotes neuronal cell death *in vitro*. Initial studies suggested relatively low LRRK2 transcript levels in neurons of the substantia nigra, with higher levels in the dopaminergic area. However, immunohistochemical analyses using available polyclonal antibodies suggest wide-spread LRRK2 protein expression in several human brain regions. Furthermore, the presence of LRRK2 protein in Lewy bodies is still controversial.

Methods: Differential centrifugation followed by standard immunoblotting. Immunohistochemistry, using formalin-fixed brain tissue, was applied to human brain post-mortem tissue obtained from the Queen Square Brain Bank. Novel monoclonal antibodies were provided by the Michael J Fox Foundation for Parkinson's Research.

Results: Initial characterisation of three monoclonal LRRK2 antibodies by immunoblotting suggests specificity on detecting the endogenous protein. Furthermore, the detection of a higher molecular weight band, mainly in the SDS-soluble fraction, suggests the presence of a possible LRRK2 protein complex associated with cellular membranes. LRRK2 protein was not detected in urea-soluble fractions.

Conclusions: Our preliminary data in human post-mortem brain tissue is in accordance with recently published research on cultured cells that suggests an association of a LRRK2 complex with cellular membranes, raising implications on the putative role of LRRK2 in membrane-initiated signal transduction pathways. The specific detection of LRRK2 protein by well-characterised antibodies will provide an imperative tool in the search for substrates and interacting partners, and also on the LRRK2 involvement in PD pathogenesis.

Exendin-4 increases acute L-DOPA-induced DA release and reduces chronic L-DOPA-induced dyskinesias

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Objective: To evaluate the effects of the glucagon-like peptide 1 agonist exendin-4 (EX-4) on acute L-DOPA-evoked striatal DA release and chronic L-DOPA-induced dyskinesias (LIDs) in 6-hydroxydopamine (6-OHDA) lesioned rats.

Background: L-DOPA is the mainstay treatment for Parkinson's disease (PD), but has severe side effects including movement disorders, i.e. LIDs. EX-4 reverses key deficits in pre-clinical rodent models of PD. In the present study, we have investigated whether treatment with EX-4 can modify L-DOPA-induced striatal DA release as well as the development of LIDs.

Methods: Male Wistar rats were lesioned with 6-OHDA and after seven days administered EX-4 (0.5µg/kg) or vehicle daily for another seven or 14 days. Thereafter, in acute L-DOPA experiments, rats were implanted with dialysis probes into the striatum and dialysed to assess the effect of L-DOPA on extracellular DA. In other experiments seven days post 6-OHDA, L-DOPA was given twice daily (10mg/kg) for 14 days concomitantly with EX-4 and LIDs scored at two, four, seven, 10 and 16 days (see Monville et al, *Brain Res. Bull* 78, 248–253).

Results: 6-OHDA greatly reduced basal extracellular DA, as well as L-DOPA-induced DA release, which was reversed by EX-4. Chronic L-DOPA rapidly induced LIDs that were progressively decreased over time by EX-4.

Conclusions: These data, coupled with our previous preclinical studies with EX-4, suggest that by normalising the functional integrity of the nigrostriatal system, EX-4 increases L-DOPA-evoked DA release in lesioned rats. This suggests that EX-4 treatment in patients could allow the use of lower L-DOPA doses to the same therapeutic effect, while decreasing L-DOPA side effects. Moreover, EX-4 may be able to reduce LIDs, which would greatly improve life quality during L-DOPA treatment.

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P100 Endocannabinoids potentiate MPP+ toxicity in a cell culture model of PD

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Objective: To investigate whether endocannabinoids exert a protective effect in a cell culture model of PD.

Background: We have established a cell culture model of PD in which we have demonstrated a protective effect of the phytocannabinoid delta-9 tetrahydrocannabinol (Δ^9 THC). The endocannabinoid anandamide (AEA) has been shown to be upregulated in PD. Here, we investigated whether the endocannabinoids, AEA and 2-arachidonylglycerol (2AG), also resulted in neuroprotection.

Methods: SHSY5Y cells were differentiated with retinoic acid and exposed to the neurotoxin, 1-methyl-4-phenylpyridine (MPP⁺). The presence of enzymes involved in endocannabinoid metabolism was determined using RT-PCR, immunohistochemistry and Western blotting. Endocannabinoids and modulators of the endocannabinoid system were co-administered with MPP⁺ and cell death assessed by the LDH assay.

Results: We demonstrated the presence of enzymes involved in the synthesis and breakdown of the endocannabinoids AEA and 2AG in differentiated SHSY5Y cells. We found that although the exogenous application of endocannabinoids by themselves was not toxic to the cells, their co-application with MPP⁺ potentiated cell death. Furthermore, inhibition of 2AG breakdown by the specific monoacylglycerol lipase (MAGL) inhibitor, JZL184, as well as inhibition of the anandamide uptake transporter by AM404 significantly potentiated MPP⁺-induced neurotoxicity. In contrast, the fatty acid amide hydrolase (FAAH) inhibitors, URB597 and JNJ1661010, which inhibit the breakdown of AEA, exerted a protective effect.

Conclusions: We have demonstrated potentiation of MPP⁺-induced neurotoxicity by the endocannabinoids, AEA and 2AG, as well as inhibitors of 2AG hydrolysis and AEA uptake. This neurotoxicity of endocannabinoids apparent under conditions of oxidative stress, such as occurs with MPP⁺, may be of relevance to the pathogenesis of idiopathic Parkinson's disease, in which upregulation of endocannabinoids has been demonstrated.

Role of CD200R, matrix metalloproteinases 3 and 9 in triggering the inflammatory response in the substantia nigra of 6-hydroxydopamine model of Parkinson's disease

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Objective: Assess the role of MMP3/9 and CD200R in microglial activation in the 6-OHDA model of PD.

Background: Microglial activation in the substantia nigra (SNc) is proposed to play an important role in dopaminergic (DA) neurodegeneration in Parkinson's disease (PD). Limited information exists on triggering signals that activate microglia. In homeostasis, local interaction between the microglia receptor CD200 (CD200R) and its neuronally expressed ligand CD200 maintains microglial quiescence. Signalling molecules, such as matrix metalloproteinases (MMPs), are proposed to be involved in microglial activation following CNS insults.

Methods: We investigated a time course of MMP-3, MMP-9 or CD200R expression in relation to microglial activation and how this correlates with SNc DA neurodegeneration in the 6-hydroxydopamine PD model. Expression patterns of MMP-3, 9, CD200R in relation to microglial (OX-6 and CD-68 expressing cells) activation was assessed one, three, five, seven and nine days after 6-OHDA lesion induction.

Results: Neuronal expression of MMP-3 and MMP-9 in the SNc occurred rapidly after 6-OHDA lesioning and preceded significant microglial activation and DA neuronal cell loss, which occurred on days seven and nine respectively. CD200R+ve cells were attached to the cell bodies and dendrites of dopaminergic neurons from seven to 15 days after 6-OHDA lesion induction, indicating a dysregulation in neuron-glia interaction, contributing to microglial activation.

Conclusion: This study clearly demonstrates that both MMP-3 and MMP-9 and subsequently CD200R are involved in microglial activation in the 6-OHDA model of PD. Such factors may be suitable drug targets to prevent microglial activation.

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Neuroprotection/neurorestoration by the flavonoids Tangeretin and Prevesteine in the 6-OHDA model of Parkinson's disease

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Objectives: Investigate the neuroprotective effects of the flavonoids Tangeretin and Prevesteine.

Background: The neurodegenerative processes precipitating Parkinson's disease (PD) are thought to be a complex interplay between factors including oxidative stress, iron accumulation, altered proteins, inflammation, etc. Flavonoids are natural products possessing several cytoprotective functions, including as antioxidants, chelate metals, anti-inflammatory, stimulation of anti-oxidant enzymes and protection against alpha-synuclein toxicity. Previously, we demonstrated that the flavonoid Tangeretin was neuroprotective when administered at the same time as a 6-OHDA lesion and for four days after. Here, we investigate whether Tangeretin and Prevestein are neuroprotective in a system that is already degenerating.

Methods: Rats received a 6-OHDA lesion to the left mfb. Seven days after 6-OHDA lesioning, Tangeretin (20mg/kg) and Prevestein (100mg/kg) (mixture of Genistein & Daidzein) was administered orally, every day for six weeks. At the end of the study, the integrity of the nigrostriatal system was assessed using tyrosine hydroxylase (TH)/NeuN immunohistochemistry in the substantia nigra and HPLC analysis of striatal dopamine. Spectroscopic techniques were utilised to investigate whether flavonoid treatment enhanced the activity of Mn and CuZn-superoxide dismutase (SOD), catalase and glutathione peroxidase activity.

Results: 6-OHDA lesioning produced a ~50% reduction in TH+ve neurons and a 64% reduction in striatal dopamine after seven weeks. Chronic treatment with both Tangeretin and Prevestein for six weeks starting one week after lesioning attenuated the neuronal loss by ~30% and loss of striatal dopamine by ~24%. Either flavonoid had no effect on the activity of Mn or CuZn SOD catalyse, but significantly increased glutathione peroxidase activity.

Conclusion: Both Prevestein and Tangeretin are neuroprotective/neurorestorative in an already degenerating nigrostriatal system, which supports their potential use in clinical trials.

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Food restriction in the 6-OHDA lesioned rat has long term consequences on L-DOPA responding

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Objective: As a precursor to evaluating the effects of L-DOPA on operant behaviour, this study examined the impact of food restriction and priming on the response to L-DOPA.

Background: L-DOPA competes with dietary amino acids for LAT2-mediated transport across the intestinal wall. Rodents used in L-DOPA studies are typically on ad libitum food as subcutaneous (sc) and intraperitoneal injections circumvent any major obstacles of gut absorption. However, competition also occurs at the LAT1 transport system on the blood-brain barrier (BBB). The present experiment therefore examined the impact of food restriction, which is common in operant studies, on the functional and dyskinesogenic effects of L-DOPA in the 6-OHDA rat model of PD.

Methods: 6-OHDA lesioned rats, maintained on either free or restricted food intake, were administered seven s.c. L-dopa doses in a pseudorandomised order. The rotational response to L-dopa before and after chronic treatment was measured using rotometers. Dyskinesia was assessed using a standardised scoring system. ¹

Results: Food restriction increased the rotational response to L-DOPA both before and after priming. Restricted food intake during initial exposure also increased the severity of dyskinesia during priming, despite all animals being on ad libitum food during the chronic treatment.

Conclusions: The experiment demonstrated that food restriction:

- significantly alters the behavioural response to sc L-DOPA treatment, suggesting a central effect, which we hypothesise is attributable to competition with dietary amino acids for BBB transport
- if occurring during initial L-DOPA exposure, may have long-term effects on subsequent dyskinesia severity
- requires an adjustment of the administered dose

References:

- Winkler, C et al (2002) 'L-DOPA-induced dyskinesia in the intrastriatal 6-hydroxydopamine model of Parkinson's disease: relation to motor and cellular parameters of nigrostriatal function', *Neurobiology of Disease*; 10(2):165-186.

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PD, masculinity and ageing: exploring the lived experiences of men living with Parkinson's disease

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Objective: To examine the lived experience of men suffering from Parkinson's disease (PD), the roles of male gender identity and experiences of the ageing process in contributing to these lived experiences, and their implications for PD sufferers and clinical practice.

Background: PD is an illness commonly affecting older men, leading to profound negative effects on quality of life. Men's concerns about PD and its impact on male gender identity, how they see themselves as men and their experiences of the illness in terms of the ageing process have only rarely been considered. But these are likely to play a significant role in men's individual experience of the illness.

Methods: This study undertakes a qualitative phenomenological investigation of 15 men suffering from PD. Participants were selected from patients taking part in the PROMS PD research study. Participants were purposively sampled according to age, range of PD symptoms and severity of disease. Each participant took part in two qualitative interviews. Interviews were transcribed and analysed using thematic analysis.

Results: This presentation communicates early results of a thematic analysis of qualitative narrative interviews. The impacts of PD on an individual's experience of the body, of temporality, of lived space, and of interpersonal relationships are discussed. Leading from these, PD leads to profound disruptions to male self identity, shaped by experiences of the ageing process and judged through individuals' positions at differing stages of the lifecourse.

Conclusions: Men's lived experiences of PD are mediated through their social status as men of differing ages. The implications of these experiences for PD patients and for wider clinical practice with men suffering from PD are discussed.

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