

## **List of proposed projects (15+1)**

**3 month rotation project:**

**Supervisor: Dr Rina Bandopadhyay**

**Reta Lila Weston Institute of Neurological Studies, UCL Institute of Neurology,**

**1, Wakefield Street, WC1N 1PJ.**

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### **Investigation of beta-synuclein gene expression in Parkinson's disease**

Parkinson's disease (PD) is a multifactorial disease that appears to be caused by genetic and environmental factors. Beta-synuclein constitutes together with alpha-synuclein and the gamma-synuclein family, a group of homologous small proteins. Missense mutations and multiplications in the alpha-synuclein gene are associated with autosomal dominant PD and it is also a major component of Lewy Body inclusions. An increasing amount of evidence supports a role for beta-synuclein in modulating alpha-synuclein aggregation and toxicity (1,2,3).

Beta-synuclein is highly homologous to alpha-synuclein but lacks a portion of the central hydrophobic residues and exhibits the properties of a random coil. We are currently studying the various alpha-synuclein isoform expressions in PD. We hypothesise that an imbalance between alpha and beta-synuclein expressions are linked to the pathogenesis of PD. So far very little is known about beta-synuclein expression in human brain.

**Aim:** To investigate the beta-synuclein gene expression in PD.

**Materials and Methods:** We will use human brain material from the QSBB archive. Using qPCR methods, we will investigate beta-synuclein gene expression in substantia nigra, striatum, and frontal cortex (some of the regions that are involved in PD pathogenesis) from neurologically normal and idiopathic PD cases. Data will be normalised to housekeeping genes. An n of 20 cases will be studied.

**References for further reading:**

1. Hashimoto M et al, 2001, Neuron, 32, 213-223.
2. Uversky VN et al, 2002, JBC, 277: 11970-11978.
3. Fan Y et al, 2007, Hum Mol Genet. 15: 3002-3011.

**Project title: Understanding Mal de Debarquement Syndrome.**

**Supervisor: Prof Brian L Day**

Mal de Debarquement Syndrome (MdDS) gets its name from its manifestation after an extended sea voyage, although this is not always the case. It is characterised by almost continuous illusory self-motion when at rest and the symptoms subside only when the sufferer is back in motion. MdDS is a debilitating condition that has no known physical cause. The vestibular system has often been suggested as the likely source of the problem but neurological and vestibular examinations usually are normal. Little is known about the syndrome and the underlying pathophysiology is a complete mystery. While all sufferers have sensations of movement, only some appear to have problems with their balance. This project aims to measure and characterise the site, size, direction and waveform of the illusory movements felt by the sufferer and relate it to their balance performance. The questions we will ask are: 1) do all sufferers experience the same illusory movements; 2) Are the illusory movements referenced to the head or the body; 3) Are balance problems associated with a particular pattern of illusory movement? These questions will be investigated using 3-D motion capture equipment in the Whole-Body Sensorimotor Lab.

## Voltage gated calcium channels in disease, role of $\alpha_2\delta$ subunits

### PhD student rotation/PhD project 2009-10

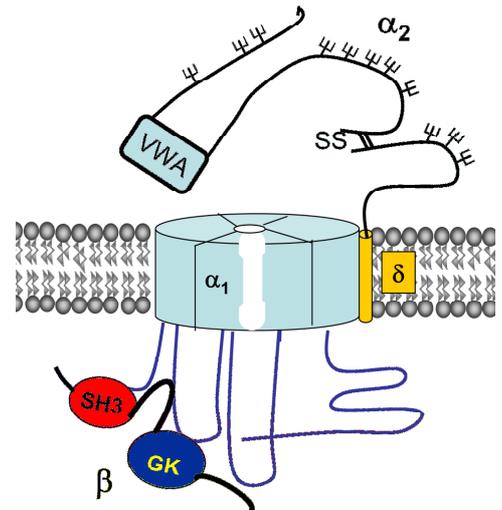
Prof. Annette C. Dolphin, Dept of Neuroscience, Physiology and Pharmacology, Andrew Huxley Building, Gower St. Campus, UCL.

contact [a.dolphin@ucl.ac.uk](mailto:a.dolphin@ucl.ac.uk) or 207 679 3276 (internal 33276). See also my personal website <http://www.ucl.ac.uk/~ucllado/> where you can also download our papers).

### Background

❖ The  $\alpha_1$  subunits of voltage-gated calcium channels are the pore-forming subunits. The accessory  $\beta$  subunits are cytoplasmic, whereas the  $\alpha_2\delta$  subunits are largely extracellular. Both accessory subunits have profound effects on calcium channel expression and properties.

❖ In my lab we work on several main areas including the role of the calcium channel  $\alpha_2\delta$  subunits and their relation to disease. The  $\alpha_2\delta$ -1 and  $\alpha_2\delta$ -2 proteins bind to the anti-epileptic and anti-nociceptive drug gabapentin. The projects relate to this area, which has been funded by Wellcome Trust, MRC and BBSRC grants.



### Three month project placement and PhD outlines

### Example Project: Alternative splicing of $\alpha_2\delta$ subunits in neuropathic pain and epilepsy models

#### Aims

There are several known splice variants and SNPs of  $\alpha_2\delta$ -1 (gene name *CACNA2D1*). We know that  $\alpha_2\delta$ -1 mRNA and protein is up-regulated in neuropathic pain. You will now investigate if the mRNA for  $\alpha_2\delta$ -1 undergo differential alternative splicing under neuropathological conditions. This might underlie differential responses to gabapentin.

#### Methods

You will investigate this initially by quantitative PCR whether there is a differential up-regulation of certain of the splice variants of  $\alpha_2\delta$ -1 in neuropathic pain. If expanded into a PhD, you will also investigate the functionality of the protein products, in terms of gabapentin binding and electrophysiological properties.

### 3 MONTH ROTATION PROJECT FOR 4 yr Clinical Neurosciences PhD programme 2009-10

(1) Title of Project: **The neurobiology of pain in juvenile arthritis**

(2) Supervisors: **Professor Maria Fitzgerald**, (m.fitzgerald@ucl.ac.uk)

UCL Neuroscience, Physiology & Pharmacology

**Dr Suellen Walker**, (suellen.walker@ich.ucl.ac.uk)

Clinical Senior Lecturer in Paediatric Anaesthesia & Pain Medicine, ICH

(2) Summary of work to be done

Each year about 10 in 100 000 children develop inflammatory arthritis. Pain is the most common reported symptom in this disease (Kimura & Walco, 2007) and is a more important factor in disability than the progression of the disease itself (Woolfe, 2000, Oen et al 2003b). Just a small decrease in pain intensity has been shown to have an appreciable effect on these children's well-being (Dhanani et al, 2007). A striking and especially difficult feature of this pain is that it spreads into the unaffected as well as the affected joints and persists even when the inflammation itself is resolved (Hogeweg, 1995a&b, Jeppesen et al., 2008).

Our lab specializes in the neurobiological basis of developing pain pathways and we have shown, using neurophysiological and behavioural methods that the normal maturation of CNS pain processes depends upon balanced sensory experience (Fitzgerald, 2005 & see (<http://www.ucl.ac.uk/npp/mfi.html>)). Nociceptive circuits are not fixed or preset at birth, but are in a plastic or transitory stage, responsive to the sensory experience which makes the system especially vulnerable to aberrant input. We hypothesise therefore that the persistent pain of juvenile arthritis results from long term sensitization of central nociceptive pathways within the CNS, triggered by excessive noxious inputs from inflamed joints at a critical stage of early life.

The purpose of this project is to investigate the mechanisms underlying the widespread pain of juvenile arthritis that persists beyond the period of active inflammation. The rotation project will involve setting up an effective animal model of juvenile inflammatory joint pain where inflammation of joints in early life causes acute central sensitization that spreads beyond the damaged area and outlasts the period of inflammation. This will involve developing skills in small animal surgery, behavioural sensory testing and 'in vivo' nociceptive reflex electrophysiology\*.

This project could be stand alone or lead on to a PhD where we will test whether

(i) Inflammation of joints in early life results in an acute immune cellular response and release of pro-nociceptive cytokines in the spinal cord which differs from that seen following adult joint inflammation.

(ii) Inflammation of joints in early life also results in an a chronic alteration in nociceptive processing such that animals are more sensitive to painful stimulation in adult life

(iii) The acute and chronic effects of early joint inflammation upon pain sensitivity is resistant to anti-TNF and methytrexate therapy in early life.

\* This project requires a Home Office animal licence, which can be arranged with 3 months advanced warning.

## **PhD student short project outline**

**Supervisors: Dr Janice Holton & Professor Caroline Sewry**

**Title: Inclusion body myositis: The 'Alzheimer's disease of muscle' – a diagnostic challenge**

### **Background**

Sporadic inclusion body myositis (IBM) was first described in 1967 and is now recognised as the most frequently occurring inflammatory myopathy in patients aged over 50 years. Unlike the other major forms of idiopathic inflammatory myopathy, polymyositis (PM) and dermatomyositis (DM), IBM responds very poorly to medications aimed at modulating the inflammatory response and the disease pursues a relentless clinical course and may result in severe disability. Histological examination of muscle biopsies from patients with IBM reveals variable inflammatory changes coupled with the presence of characteristic rimmed vacuoles. There may also be additional abnormalities with evidence of denervation and increased ragged red and/or cytochrome oxidase negative fibres. Ultrastructural studies have revealed that rimmed vacuoles are composed of whorled membranous debris together with tubulo-filamentous inclusions 15-21nm in diameter and that such tubulo-filamentous inclusions may also be found in myonuclei (1). More recent studies employing immunohistochemical methods have demonstrated that rimmed vacuoles contain a number of proteins commonly associated with neurodegenerative diseases such as amyloid  $\beta$  ( $A\beta$ ), its precursor protein  $\beta$ APP,  $\alpha$ -synuclein, phosphorylated tau, and prion protein, with ultrastructural studies identifying the accumulation of amyloid and paired helical filaments (2-4). These findings have led to the analogy with Alzheimer's disease and it is currently debated whether inflammation or degeneration is the predominant mechanism underlying IBM (5-7).

Current diagnostic criteria for IBM include clinical and pathological features. The most recent pathological criteria require the identification of endomysial inflammation with infiltration of intact myofibres, rimmed vacuoles and either amyloid or characteristic tubulo-filamentous inclusions on ultrastructural examination (8). More recently these criteria have been modified to include other features such as increased MHC Class I expression on muscle fibres (a marker of an inflammatory process), and cytochrome oxidase negative fibres (7). However, despite a number of publications detailing the accumulation of abnormal proteins and the presence of amyloid in muscle fibres in IBM a systematic study to identify the most sensitive method of demonstrating abnormal protein accumulation has not been performed and it is also unclear whether the addition of immunohistochemical staining may assist in differentiating IBM from other myopathies with rimmed vacuoles such as the myofibrillar myopathies. It is also apparent that a unified approach to the pathological diagnosis of IBM is not applied in the UK (MRC Centre for Neuromuscular Diseases IBM Workshop 13<sup>th</sup> June 2008). It is therefore timely to examine these issues and propose updated pathological criteria for the diagnosis of IBM taking into account the recent immunohistochemical

data and applying techniques readily performed in any UK diagnostic pathology laboratory. Such an approach will permit a uniform approach to diagnosis which is critical for patient management and accurate patient selection and entry into future clinical trials of therapy in IBM.

### **Specific aims**

1. To identify the most sensitive method(s) of detecting protein accumulation(s) in rimmed vacuoles.
2. To determine if, and what type of, protein accumulation(s) can be detected in the absence of rimmed vacuoles.
3. To identify the most sensitive method(s) for differential diagnosis of IBM from other myopathies with rimmed vacuoles, such as myofibrillar myopathies and hereditary IBM.
4. Propose revisions to the Griggs diagnostic criteria for IBM.

### **Plan of investigation**

#### *Material and methods*

Six cases of clinically and pathologically defined IBM will be selected from the archives of the Division of Neuropathology. Cases will be chosen in which there is adequate frozen tissue available for study and these will form the cohort for the study. Further well defined cases of DM, PM, necrotising myopathy, myofibrillar myopathy and normal controls will also be identified for study.

Frozen neocortical tissue from cases of Alzheimer's disease and cortical Lewy body disease will be requested from the Queen Square Brain Bank for Neurological Disorders.

Frozen sections of brain tissue and muscle tissue will be cut using a Bright cryostat.

Amyloid staining will be performed using routine laboratory procedures for Congo red, thioflavin S and crystal violet.

Immunohistochemical staining will be performed using a standard avidin-biotin complex protocol and antibody binding sites will be visualised using the chromogen diaminobenzidine.

*Specific aim 1: To identify the most sensitive method(s) of detecting protein accumulation(s) in rimmed vacuoles.*

Methods for staining amyloid will be optimised in frozen tissue sections from a case of Alzheimer's disease and a case of cortical Lewy body disease. Methods to be used are Congo red, thioflavin S and crystal violet. After optimisation of the laboratory procedure the methods will be applied to the cohort of 6 IBM cases. The most sensitive method will be determined by assessing the percentage of fibres containing amyloid deposits with each staining method.

*Specific aim 2: To determine if, and what type of, protein accumulation(s) can be detected in the absence of rimmed vacuoles.*

A number of different proteins commonly associated with neurodegenerative diseases have been identified in protein aggregates in muscle fibres in IBM. It is unclear whether these are only present in identifiable rimmed vacuoles or whether they can also be detected in muscle fibres without vacuoles. The most sensitive method for detecting protein aggregates in skeletal muscle will be determined by immunohistochemical staining of several proteins including phosphorylated tau, A $\beta$ , APP,  $\alpha$ -synuclein, TDP-43, VCP, lamin A/C, emerin,  $\alpha$ Bcrystallin in the cohort of 6 IBM cases. Serial areas will be identified and the percentage of labelled fibres assessed to determine which proteins are aggregated and represent the most sensitive method of detecting abnormal protein accumulation in this condition.

*Specific aim 3: To identify the most sensitive method(s) for differential diagnosis of IBM from other myopathies with rimmed vacuoles, such as myofibrillar myopathies and hereditary IBM.*

The staining protocols developed in specific aims 1 and 2 will be applied to a group of cases of IBM, DM, PM, normal controls (minimum 10 cases of each). Cases of necrotising myopathy and myofibrillar myopathy will also be included if possible. The purpose of this is to determine which stains may be helpful in distinguishing between conditions which have similar pathological appearances when routine staining methods are applied.

*Specific aim 4: Propose revisions to the Griggs diagnostic criteria for IBM.*

The final outcome from this study will be to propose a modification of the currently used criteria for the pathological diagnosis of IBM. It is anticipated that the approach suggested will only involve methods widely available in pathology laboratories and the aim is to assist in providing a uniform approach to the diagnosis of this condition

### **Project location, facilities and support**

The student will work in the Division of Neuropathology, Institute of Neurology where desk space and computer access will be provided. The student will join the teams involved in the diagnosis of

adult and paediatric muscle disorders. Together these teams form one of the largest muscle diagnostic facilities in the UK. Experienced members of the team will be available to provide training and on-going support for the student in all required diagnostic techniques. Where appropriate, additional training will be provided by staff from the Queen Square Brain Bank for Neurological Disorders. The Division of Neuropathology has all of the equipment required for this project. The student will be encouraged to attend the weekly multidisciplinary team meetings held by the adult and paediatric muscle pathology teams and any other relevant muscle pathology meetings.

### **Expected outcome**

It is expected that the student will complete the laboratory work outlined within the three months allocated for this project. This will enable the student to make a major contribution to the preparation of a manuscript to be submitted for publication. As the content of the manuscript will be a proposal for revised pathological criteria for the diagnosis of IBM it is expected that such a paper would be of high impact and frequently quoted in the literature.

### **Requirements**

**N.B.** The student must be able to demonstrate that they have been immunised against hepatitis B and have adequate immunity before laboratory work can commence.

### Reference List

- (1) Dubowitz V, Sewry C. Inflammatory myopathies. *Muscle Biopsy A Practical Approach*, 3rd ed Saunders Elsevier, 2007:519-539.
- (2) Maurage CA, Bussiere T, Sergeant N, et al. Tau aggregates are abnormally phosphorylated in inclusion body myositis and have an immunoelectrophoretic profile distinct from other tauopathies. *Neuropathol Appl Neurobiol* 2004 Dec;30(6):624-634.
- (3) Askanas V, Engel WK. Molecular pathology and pathogenesis of inclusion-body myositis. *Microsc Res Tech* 2005 Jul;67(3-4):114-120.

- (4) Askanas V, Engel WK. Inclusion-body myositis, a multifactorial muscle disease associated with aging: current concepts of pathogenesis. *Curr Opin Rheumatol* 2007 Nov;19(6):550-559.
- (5) Murphy MP, Golde TE. Inclusion-body myositis and Alzheimer disease: two sides of the same coin, or different currencies altogether? *Neurology* 2006 Jan 24;66(2 Suppl 1):S65-S68.
- (6) Dalakas MC. Sporadic inclusion body myositis--diagnosis, pathogenesis and therapeutic strategies. *Nat Clin Pract Neurol* 2006 Aug;2(8):437-447.
- (7) Needham M, Mastaglia FL. Sporadic inclusion body myositis: a continuing puzzle. *Neuromuscul Disord* 2008 Jan;18(1):6-16.
- (8) Griggs RC, Askanas V, DiMauro S, et al. Inclusion body myositis and myopathies. *Ann Neurol* 1995 Nov;38(5):705-713.

## **Modulating volition with direct current stimulation of medial frontal cortex**

**Patrick Haggard, Institute of Cognitive Neuroscience, UCL**

[p.haggard@ucl.ac.uk](mailto:p.haggard@ucl.ac.uk)

Several neurological and neuropsychiatric syndromes suggest a critical role for the medial frontal cortex in the initiation of voluntary action. However, the lack of objective and sensitive measures of human volition has limited development of assessments and therapies for disorders involving this area. Two potentially sensitive markers of volition are the perceived time of intentions preceding actions, and the ability to prepare an action but then to inhibit (“veto”) it at the last possible moment. This project will investigate the contribution of medial frontal circuits to both these aspects. The student will deliver anodal (enhancing) and cathodal (inhibiting) transcranial direct current stimulation to healthy volunteers in separate sessions, while measuring behavioural and conscious markers of voluntary action. A potential extension would involve measuring the effects of tDCS on preparatory EEG activity preceding voluntary action. The results could have translational benefit in suggesting potential therapies for disorders of voluntary action.

### **References**

- 1 Haggard P (2008). Human volition: towards a neuroscience of will. *Nature Reviews Neuroscience*, 9, 934-946.
- 2 Brass M & Haggard P. (2007). To do or not to do : the neural signature of self-control. *Journal of Neuroscience*, 22, 9141-9145.

## **Blakemore Lab projects**

The ICN Developmental Cognitive Neuroscience Group focuses on the development of mentalising, emotions, action understanding and executive function during adolescence. A second focus of our research is on social cognitive deficits in autism spectrum disorders. Our research involves a variety of behavioural (psychophysics, eye-tracking, motion capture) and neuroimaging (MRI, fMRI and MEG) methods.

**Group Leader:** [Sarah-Jayne Blakemore](#)

<http://sites.google.com/site/blakemorelab/>

### **Rotation project 1: the social brain in adolescence**

The peak age of onset for many psychiatric disorders is adolescence. In the past decade or so, a number of structural magnetic resonance imaging (MRI) studies have shown that, in humans, several brain regions undergo substantial changes in white matter and grey matter density during the first two decades of life. Some of the brain regions that are late-maturing include parts of the “social brain”, that is, the network of brain regions that is used to understand and interact with other people. We use novel social cognition tasks that tap into how you understand other people’s minds and emotions. We look at social brain development using a variety of methods. A behavioural study would involve testing children and adolescents in schools. Alternatively you could get involved in ongoing functional neuroimaging (fMRI or MEG), eye-tracking or movement tracking studies. This research has potential clinical implications because many psychiatric disorders which have their onset during adolescence.

#### References

Blakemore SJ. The social brain in adolescence. *Nat Rev Neurosci.* 9(4):267-277 (2008).

Paus T, Keshavan M, Giedd JN. Why do many psychiatric disorders emerge during adolescence? *Nat Rev Neurosci.* 9(12):947-957 (2008)

### **Rotation project 2: Social cognition in autism spectrum conditions**

Autism spectrum condition (ASC) is a pervasive developmental disorder characterised by difficulties with reciprocal social interactions in addition to unusual patterns of repetitive behaviour and verbal and non-verbal communication problems. ASCs affect up to 1% of the population and social interaction impairments significantly impact on the quality of these individuals’ lives. We are interested in how the social brain functions in ASC. We use a variety of novel tasks that tap into social cognition and investigate social cognitive function in ASC either behaviourally, or using functional neuroimaging (fMRI or MEG), eye-tracking or movement tracking.

## References

Boraston, Z, Blakemore, S-J, Chilvers, R, Skuse, D. Impaired sadness recognition is linked to social interaction deficit in autism. *Neuropsychologia* 45(7), 1501-1510 (2007)

Viding, E, Blakemore, S-J. Endophenotype approach to the study of developmental psychopathology: implications for autism. *Behavior Genetics* 37(1), 51-60 (2007)

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<http://sites.google.com/site/blakemorelab/publications>

## Decision-making in major depression

**Dr Jonathan Roiser, Institute of Cognitive Neuroscience**

[j.roiser@ucl.ac.uk](mailto:j.roiser@ucl.ac.uk)

**Background:** Depression is a common and debilitating disorder whose cause is poorly understood. Although mood abnormalities are the classic hallmarks of the disease, cognitive dysfunction is also a core feature of its pathophysiology. In particular, healthy individuals may gain resilience from depression by evaluating the world in an optimistically biased manner, while individuals who exhibit a more realistic cognitive style may be predisposed to the disease, a feature known as “depressive realism”. In a recent computational model, Dayan and Huys (2008) proposed that this difference between healthy and depressed individuals partly stems from the availability of serotonin, which in their model causes people to reflexively inhibit behaviours predicted to have adverse consequences. According to this model, individuals with normal serotonin levels reflexively “prune” away series of options with poor expected outcomes, thus under-exploring the environment and biasing their valuations of the environment. Although such pruning may be adaptive, any drop in serotonin levels (e.g., preceding the onset of depression) would compromise this reflexive inhibition and cause individuals to experience a greater number of surprising adverse events.

**Project outline:** As a first step towards testing the Dayan and Huys model, we have devised a behavioural task in which volunteers make chains of decisions for monetary reward and punishment. Using computational techniques, we are able to calculate how pruning affects behaviour on this task, causing systematic performance differences between healthy and depressed volunteers. In our pilot study, we have found that healthy volunteers exhibit significant pruning, and that their level of pruning correlates inversely with anxiety and neuroticism, as directly predicted by the model. The aim of this rotation project is to extend this investigation to a sample of unmedicated depressed patients and matched controls, with the goal of better understanding the role of pruning in the development or maintenance of the major depressive disorder.

**Extra considerations:** Since this project involves testing currently depressed patients, the student carrying out the project will need to have a Criminal Records Bureau check. This is a straightforward administrative procedure carried out by the NHS trust, but can take a few weeks. Therefore, potentially interested students should contact Dr Jonathan Roiser as soon as possible.

## **Project title: Cognitive control of attention in prefrontal cortex**

**Nilli Lavie**

### Summary:

Distractibility, manifested in inappropriate responses to salient but irrelevant stimuli, is a prominent symptom of patients with a prefrontal lesion. This is often attributed to the deficits in cognitive control functions such as “working memory” that tend to also follow prefrontal lesion. However, it is hard to infer any direct causal role merely from the co-occurrence of symptoms after a large anterior lesion.

In the projects offered we attempt to better understand the deficits following a prefrontal lesion and establish a causal role for prefrontal cognitive control functions in these failures of attention.

In one project we use TMS to temporarily disrupt prefrontal activity in the intact human brain (i.e. apply TMS over prefrontal cortex of healthy volunteers), during performance in cognitive tasks that measure attention (we use for example measures of “inattention blindness”) and conversely distraction.

This project would allow us to establish a causal role for particular parts of frontal cortex (e.g. the Anterior Cingulate) in attention failures.

In another project, we engage frontal cognitive control functions in a high- load task (e.g. a working memory task requiring rehearsal of many items) and assess the effects on the subject responses to distractors.

This project would allow us to establish a causal role for particular frontal cognitive control functions (e.g. working memory) in attention failures.

## Investigating the centrality of the Golgi apparatus to juvenile neuronal ceroid lipofuscinosis

Laboratory of Dr Sara E Mole, MRC LMCB, UCL

The neuronal ceroid lipofuscinoses (NCL, or Batten disease) are a group of so far untreatable diseases that are the most common neurodegenerative diseases of childhood, characterised by lysosomal storage. The prevalent type is juvenile NCL, which is caused by mutations in *CLN3* that encodes a polytopic membrane protein. We recently showed that the most upstream intracellular effect of loss of *btn1*, the fission yeast orthologue of *CLN3*, is aberrant Golgi morphology and a defect in trafficking of the Vps10p receptor for carboxypeptidase Y (CPY) (Codlin and Mole, 2009). This finding is consistent with the multiple downstream effects of loss of *btn1* on other intracellular pathways that affect vacuole size and pH, cell wall structure and deposition, and polarised growth (Gachet et al., 2005, Codlin et al, 2008a, Codlin et al., 2008b), all of which are rescued by expression of the human gene *CLN3*. These defects are differentially rescued by expression of Btn1p modelling mutations of *CLN3* that have different effects on disease severity (Haines et al 2009). It is also consistent with the primary location of Btn1p being at the Golgi apparatus (Codlin and Mole, 2009).

To further understand the contribution of Btn1p to Golgi function, electron microscopy will be used to study Golgi morphology in a series of yeast strains (e.g that include those either lacking or overexpressing *btn1* or *CLN3*). The results of this investigation should confirm and extend the importance of Btn1p for Golgi morphology and function, perhaps relating aberrant morphology with disease severity, and extending the basis for the development of future therapeutic strategies.

### References:

- Codlin, S., Haines, R. L. and Mole, S. E. (2008). *btn1* affects endocytosis, polarisation of sterol-rich membrane domains and polarised growth in *Schizosaccharomyces pombe*. *Traffic* 9, 936-950.
- Codlin, S., Haines, R. L., Burden, J. J. E. and Mole, S. E. (2008). *btn1* affects cytokinesis and cell wall deposition by independent mechanisms, one of which is linked to vacuole pH dysregulation. *J. Cell Sci.* 121, 2860-2870.
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- Gachet, Y., Codlin, S., Hyams, J. S. and Mole, S. E. (2005). *btn1*, the fission yeast homologue of the human Batten disease gene, *CLN3*, regulates vacuole homeostasis. *J. Cell Sci.* 118, 5525-5536.
- R.L. Haines, S. Codlin and S.E. Mole. 2009. *The fission yeast model for the lysosomal storage disorder Batten disease predicts disease severity caused by mutations in CLN3*. *Dis. Models Mech.* 2: 84-92.

## ***Identification of new components in HtrA2/PINK1 signalling pathways***

**Department of Molecular Neuroscience**

**Line manager: Dr H el ene PLUN-FAVREAU**

The identification of genes responsible for mendelian forms of Parkinson's Disease (PD) has transformed our understanding of the molecular pathogenesis of neurodegeneration. Whether these mendelian genes interact, and which signalling pathways are disrupted in PD remain unknown. Work in the laboratory is focusing on trying to characterise molecular pathways that appear to be of major significance in PD.

The focus of the project is to try and find out new components in molecular pathways which lead to PD. To do this we have chosen a range of molecular biological, live microscopy and cellular techniques to provide a list of new proteins. We will follow this up by assessing how these proteins talk to each other and how important these crosstalk are in the development of the disease. To this end we will have access to human brain tissue as well as to cell models and mice in which the PD mendelian genes have been disrupted.

If we can indeed discover more of the pathway to cell dysfunction and death, then this in turn provides more options for therapeutic intervention.

## Using *In Vivo* Confocal Microscopy To Identify Mechanisms Responsible For Axonal Degeneration In A Model Of Multiple Sclerosis

**Prof. Kenneth Smith, Department of Neuroinflammation, Institute of Neurology**

Axonal degeneration is a major cause of permanent disability in multiple sclerosis (MS), but little is known about the underlying mechanisms and this limits the development of a rational therapy. Mitochondrial defects and energy insufficiency are strongly implicated in the degeneration, probably initiated by factors in the inflammatory microenvironment in which the axons are embedded: a role for nitric oxide (NO) is suspected. A leading theory is that ATP insufficiency limits activity of the sodium pump, leading to a rise in intra-axonal  $[Na^+]$ , especially if impulse activity is high. The rise in axonal  $[Na^+]$  causes the  $Na^+-Ca^{++}$  exchanger to operate in reverse, importing damaging levels of  $Ca^{++}$  to the axoplasm, thereby activating enzymes that precipitate degeneration. Partial blockade of  $Na^+$  channels protects axons from degeneration in experimental models, as predicted by the theory, but other interpretations are possible. The proposed PhD project aims to determine whether this theory is true in an experimental inflammatory model of MS, and the proposed 3 month project, described below, aims to explore whether a simplified model of inflammation causes the anticipated changes in intra-axonal  $[Na^+]$  and  $[Ca^{++}]$ . The simplified model will employ exogenous NO as a substitute for inflammation, as NO is one of the key factors produced by inflammatory cells, and NO is a potent inhibitor of mitochondrial function. We have developed techniques to observe central and peripheral axons in real time, *in vivo*, using confocal microscopy, and will use these methods to observe the consequences of NO exposure: for simplicity, initial experiments will examine excised spinal roots while experience with the techniques is obtained. We will use potentiometric fluorescent dyes to measure changes in the mitochondrial membrane potential in order to assess effects of NO on mitochondrial metabolism *in vivo*. These measurements will be made simultaneously with measurements of intra-axonal  $[Na^+]$  and  $[Ca^{++}]$  using a variety of fluorescent indicators. In this way, we will be able to establish the relationship between mitochondrial function and axonal ion balance following NO exposure. Thus, we will observe, in real time *in vivo*, the effects of experimental inflammation on axonal pathophysiology, and thereby explore the mechanisms involved in axonal damage. The experiments will be performed in the Department of Neuroinflammation at the Institute of Neurology, in collaboration with Prof. Michael Duchen, UCL.

## **Clinical Neurosciences 4yr PhD - 3 months taster project**

**Title: The effect of population and scanning parameters on a super-DTI dataset. Implications for tractography and future clinical studies.**

**Principal supervisor: Claudia Wheeler-Kingshott**

**Secondary supervisors: Olga Ciccarelli, Declan Chard, Dan Tozer**

Departments: Neuroinflammation and Brain Repair and Rehabilitation

Recently CWK developed a method for registering diffusion tensor imaging (DTI) datasets from a number of healthy subjects with the aim to generate a super-DTI dataset in MNI space. This super-DTI dataset has a very high signal to noise (SNR) ratio and high resolution (1x1x1 mm<sup>3</sup>), therefore can be used, for example, for generating white matter tracts in standard space.

It is unknown, though, whether the group of subjects used to generate the super-DTI dataset has a major effect on the final super-DTI dataset, nor whether acquisition parameters for each individual DTI scans affect the super-DTI.

We have three cohorts of healthy subjects: C1, C2 and C3. For each cohort CWK has already created three separate super-DTIs. This project aims at analysing these datasets with voxel by voxel statistical comparisons of DT maps and with tractography in order to establish the dependency of the resulting super-DTI on the chosen population and on the acquisition parameters. The outcome of this study will influence future clinical studies that use DTI.

We will divide the project into two main studies:

- 1) The super DTI from each cohort has maps of FA, MD, volume ratio (VR), axial and radial diffusivities, all derived from its eigenvalues. Comparing these maps from C1 and C2, which have been acquired on different subjects but with identical scanning parameters, will establish whether the subjects chosen affect the super-DTI created from the group. Comparing the maps from C1 and C3 or C2 and C3, which were acquired with different scanning protocols, will answer the question of whether the acquisition parameters affect the super-DTI dataset. The comparison will be done on a voxel by voxel basis using SPM or FSL.

2) The super-DTI dataset can be used to define white matter tracts representing the healthy population under investigation. The high SNR of the super-DTI dataset and its high resolution are advantageous for tractography. We plan to generate three major tracts, i.e. the corticospinal tract (CST), the optic radiation (OR) and the corpus callosum (CC), for each cohort, starting from seed points and exclusion masks identical for all groups and defined in MNI space. We will compare the tracts in terms of their properties (mean parameters along the tracts) and shapes for each cohort to confirm the results of step 1) on whether the population and scanning parameters influence these outcome.

## 4yr Clinical Neurosciences PhD course: 3-month rotation project

### Diffusion-based tractography of the spinal cord to investigate mechanisms of repair in Multiple Sclerosis

**Supervisors: O. Ciccarelli & A. Thompson**

**Background:** We have developed diffusion tensor imaging (DTI) of the spinal cord and applied it to patients with Multiple Sclerosis (MS) at the onset of an acute cervical cord relapse (1). Diffusion-based tractography of the cervical cord, which was obtained using the information contained in the DTI data, was able to provide measures that were sensitive to the acute spinal cord damage and correlated with acute disability (1). A key question that remained unanswered is whether tractography-derived measures of the main spinal cord pathways at the onset of an acute inflammatory event predict future clinical recovery. In order to answer this question, we have now carried out a longitudinal study on the same patient cohort after a cord relapse. This project requires the analysis of the imaging and clinical data that have been already acquired.

**Aims:** The aims of this project are: (i) to investigate whether tractography-derived measures of the main spinal cord pathways, such as the cortico-spinal tract, predict clinical recovery; (ii) to assess whether there are correlations between changes in the tractography-derived measures and clinical changes.

**Methods:** 14 MS patients underwent spinal cord DTI at the onset of a cervical cord relapse, resulting from a lesion at C1-C3 level, and after one, three and six months. Thirteen age-matched healthy subjects were also studied. Probabilistic tractography (2) will be performed to track the lateral cortico-spinal tracts in the lateral columns, the anterior cortico-spinal tracts and the anterior spino-thalamic fasciculi in the anterior columns, and the bilateral fasciculus gracilis and cuneatus in the posterior columns. Tractography-derived measures of these tracts, including voxel-based connectivity, which reflects fibre integrity, will be obtained at each time point. Multiple linear regression analysis will identify predictors of clinical outcome. Mixed-effect linear regression models will estimate the differences in the rate of change in tractography-derived measures between patients and controls over time, and correlations between clinical changes and radiological changes.

**Expected results:** We expect to find that higher connectivity of the tractography-derived cortico-spinal tracts at baseline is associated with better clinical outcome. In addition, there will be an association between an increase in connectivity of the same tracts over time and greater clinical recovery.

**References:** (1) Ciccarelli et al, Spinal cord spectroscopy and diffusion-based tractography to assess acute disability in multiple sclerosis, *Brain* 2007, 130:2220-2231. (2) Behrens et al, Non-invasive mapping of connections between human thalamus and cortex using diffusion imaging. *Nat Neurosci*, 6(7), 750-757.