Training and Understanding Neurodegenerative Diseases

NEUROTRAIN is an Early Stage Research Training [EST] funded through the European Union research programme FP6

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Cognitive function is the most precious of all human attributes – its loss is considered as one of the most severe and dreadful handicaps for human life quality. The prevalence of neurodegenerative diseases is increasing in the European society and will get even more prevalent in our ageing population.

The focus of this Early Stage Research Training (EST) ‘NEUROTRAIN’ is to promote training possibilities and to improve our knowledge of neurodegeneration that will lead to prevention and therapy.

Improvement in quality of life is of paramount importance for Health Care providers of all governments in the European Union. Our attention must therefore focus on the ever increasing incidence of neurodegenerative diseases, resulting from an ever increasing life expectancy. In an effort to face this challenge, training and research in neuroscience must be strengthened and intensified. In America and in Europe, the last decade of the past century was defined as the "brain decade" in biomedical research. In order to encourage the synergy between researchers coming from different scientific and humanistic backgrounds, researchers from leading centres in London, Lund, Tuebingen and Munich suggest an exciting training possibility. It is presently possible to reach an understanding of the organisation of higher cognitive functions on the basis of achievements made in different scientific fields. Such knowledge is of utmost importance in order to face the increasing impact of clinical diseases, as it allows the transfer of basic research to therapeutical applications in real time. The specific objective of this Early Stage Research Training (EST) network is the integration and networking of ten outstanding research teams in the context of a well-defined research programme that will formulate and facilitate a structured training programme for researchers in the highly-specialised field of neurodegeneration research. ‘NEUROTRAIN’ will provide a unique and cohesive but flexible framework for the training and professional development of young researchers. These interacting centres bring together a critical mass of theoretical and applied research using a multidisciplinary approach. Each centre represents an existing working unit, having its own specific expertise and research agenda. Our project objectives have been designed based on the conviction and implicit belief that treatment of neurodegenerative diseases can only be achieved through a comprehensive understanding of genes involved in neuronal development, structure and cellular function in health and disease. This will allow a simultaneous approach for a functional understanding and characterization of disease mechanisms affecting central nervous system (CNS) and retinal neurons and lead to the development of therapeutic approaches, the ultimate end-point of this research programme.
PROJECT 1  Generation of dopaminergic neural precursor cells for Parkinson’s disease (Responsible Supervisor: PD Dr. MINGER, London, UK)

The Stem Cell Biology Laboratory, led by Dr Stephen Minger, is housed in the new Wolfson Centre for Age-Related Disease at King's College London. The lab is one of the pioneering groups in stem cell biology in the UK. They were one of the first two research teams in the UK to be granted a license to derive human embryonic stem (ES) cells by the Human Fertilisation and Embryology Authority in 2002 and were the first group to successfully generate human ES cells lines and to deposit these cells into the UK Stem Cell Bank. To date, the lab has generated three unique human ES cell lines, one of which harbours the most common mutation that causes Cystic Fibrosis. We will continue to attempt to generate additional human ES cell lines, particularly disease-specific cell lines, that will provide important tools not only for fundamental pathophysiological research, but also as screening tools for new therapeutic compounds and gene targeting strategies.

In addition to our work on human ES cell derivation, much of the effort in the Stem Cell Biology Laboratory is focused on the generation of somatic (e.g. tissue-specific) stem cell populations from human ES cells that may have therapeutic benefit. We are focused on the following clinical targets where cell replacement may have important therapeutic implications: (collaborators to the right)

- Spinal Cord Damage    Prof Stephen McMahon  (KCL)
- Multiple Sclerosis    Dr Paul Felts (KCL)
- Parkinson’s Disease    Dr Susan Duty (KCL)
- Heart Damage    Dr Anthony Mathur (Bart’s Hosp)
- Type I Diabetes    Prof Peter Jones (KCL)
- Retinal Disorders    Prof Robin Ali (Inst Ophthalmology)
- Hepatic Insufficiency    KCL Liver Transplant Unit
- Joint Disorders    Dr Cosimo DeBari (KCL)
- Bone Replacement    Dr Agi Grigoriadis (KCL)
- Tooth Replacement    Prof Paul Sharpe (KCL)
- Haematopoietic Stem Cells    Prof Paul Fairchild (Oxford)

In addition, we have basic science programmes cells in the following areas:

- Lung Development/CF    Novartis Institute
- Huntington’s Disease    Prof Gill Bates (KCL)
- Stem Cell Proteomics    Prof Tony Ng (KCL)
- Stem Cell Bioprocessing    Dr Chris Mason (UCL)
- Bioethics of Human ES Cells    Prof Herbert Gottweis (Vienna),
                                Prof John Harris (Manchester),
                                Prof Christine Hauskeller (Exeter)
                                Prof Brian Salter (Norwich)
                                Prof Sarah Franklin (LSE)
- Adult Neurogenesis    Prof Clive Ballard (KCL)
                         Dr Aaron Chuang (GSK)
Relevant Publications


Prof Wood is head of department of molecular neuroscience at the Institute of Neurology, UCL, London. The Institute is closely associated in its work with the National Hospital for Neurology & Neurosurgery, a Department of the University College London Hospitals' NHS Foundation Trust, and in combination they form a national and international centre at Queen Square for teaching, training and research in neurology and allied clinical and basic neurosciences.

The Institute has seven Research Departments encompassing clinical and basic research within in each theme. The dept of Molecular Neuroscience brings a range of molecular and cellular techniques to address basic and applied scientific questions which relate to human neurological disease. The Institute employs a total of around 370 staff, occupies some 11,500sq m of laboratory and office space in five buildings, and has a current annual turnover of £24m. The Institute was awarded a Grade 5*A rating (the highest possible rating) in the 2001 Exercise, a measure of its national and international standing.

Within the institute there are extensive molecular and cellular biological resources and equipment available. These include:

- Molecular genetics- High throughout sequencing and genotyping (TaqMan)
- Molecular Biology
- Quantitative realtime PCR
- Cell culture- including confocal suites
- Neurochemistry- with expertise in mitochondrial biochemistry
- Histology and immunohistochemistry

A new state of the art proteomics facility is currently being established between the Institutes of neurology and Child health which will provide a platform of technologies for use by researchers of UCL.

These techniques and technologies will be made available to the research student to further the project based on recently identified novel genes causing Parkinson’s disease- PINK1 and LRRK2 (see publication below- Valente et al 2004, Paisan-Ruiz et al 2004). The challenge is to further understand the molecular and cellular events leading to cell degeneration. The specific aims of this project are to establish a primary human neuronal cell line and a human neuroblastoma cell line which have undergone a variety of manipulations of the PINK1 and/or the LRRK2 gene. These manipulations will include over-expression and knock-down studies using siRNA.
Training:
The Institute has a large postgraduate training programme and is also responsible for teaching inpatient undergraduate neurology to University College medical students. Academic and research links with other parts of UCL are being continuously developed. Full details of the Institute’s research and teaching activity can be found on the Institute of Neurology website at http://www.ion.ucl.ac.uk.

Specifically for this project will offer the research scientist(s) training and experience in:

- Cell culture techniques - particularly handling SHSY-5Y and human neuronal dopaminergic cell lines.
- Western blotting
- Standard and advance molecular genetic/biological techniques (as required)
- Confocal microscopy
- Quantitative RNA analysis
- Antibody characterization
- siRNA techniques

Relevant publications:


Professor Shomi S. Bhattacharya is Head of the Department of Molecular Genetics at the Institute of Ophthalmology, University College London (UCL). The Institute of Ophthalmology is a multi-disciplinary research institute with expertise in electrophysiology, psychophysics, morpho logy, molecular and cellular biology, biochemistry, histopathology, clinical ophthalmology and epidemiology. It is committed to a research programme that furthers an understanding of the eye and visual system linked with clinical investigations targeted to specific problems in the prevention and treatment of eye diseases. The department of Molecular Genetics within the Institute is fully resourced and equipped with the State-of-the-Art instrumentation for molecular biology oriented research. These include a large capacity for gel electrophoresis, automated DNA sequencing, ultracentrifugation, over 30 PCR blocks, tissue culture and radioisotope laboratories, biochemistry suite for structural and functional studies and extensive computational facility. The techniques of disease gene mapping, positional cloning, mutation analysis, tissue in-situ hybridisation, in-vitro mutagenesis and biochemical techniques for protein structure and functional analysis are in routine use in the department.

Background:
Molecular Genetics defines a field of study where in the absence of a detectable biochemical defect (true for a majority of inherited diseases), a reverse genetics approach allows the chromosomal assignment and isolation of the disease causing genes. Advent of recombinant DNA technology led to the cloning of chromosome specific DNA markers in the early 1980s. The ability to detect restriction fragment length polymorphisms (RFLPs) in the Human Genome, permitted the use of these markers as genetic tools for linkage studies in families segregating for a particular disease trait.

Inherited retinal dystrophies are a major cause of incurable blindness in the Western World. Amongst these a significant proportion is accounted for by a clinically heterogeneous group of diseases collectively known as Retinitis pigmentosa (RP). Based on pedigree analysis, all three modes of Mendelian inheritance (Autosomal dominant, Autosomal recessive and X-linked) have been observed. RP is initially characterised by night blindness and progressive loss of peripheral vision due to the loss of rod photoreceptor cells. As the disease progresses cone cells in the central retina are also involved, often leading to total blindness in later stages of the disease. The major programme of research in the lab is dedicated to the understanding of the molecular genetic and functional basis of retinal degeneration.

Training:
A comprehensive training will be offered to new candidates joining the lab in the following methods:
1. Linkage mapping using hypervariable marker systems (microsatellites, SNPs)
2. Positional cloning, physical contig mapping
3. Gene prediction software and bioinformatics
4. Mutation screening using high throughput Transgenomics WAVE machine
5. PCR based direct genomic sequencing
6. Expression analysis by RT-PCR, in situ hybridisation, confocal microscopy
7. Western blotting
8. Quantitative RNA analysis
9. Writing of lab reports and manuscripts for publication
10. Poster and seminar presentation skills

List of publications:


The research work of the Department of Medical Genetics is directed towards deciphering the pathogenesis of neurodegenerative diseases such as Parkinson’s disease, spinocerebellar ataxia, dystonia and Huntington’s disease. We have a strong background on mutation screening in large patient collections, on the search for disease modifiers, but also on protein and RNA work. To our department belong a service core facility for microarray analysis (Affymetrix based) and a core facility for the generation of transgenic animals. Having this background we generated a large number of transgenic and knock out models of neurodegenerative diseases, in particular also transgenic rat models which are in particular useful for behavioral and treatment studies. Also, we are investigating altered transcriptional pathways in the brain of these models. Finally, the gain insight into the pathogenesis of neurodegeneration we are searching for unknown interacting proteins and if these proteins modify the disease process in vitro.

Histology
- Immunocytochemistry
- Histochemistry

Microarray analysis (Affymetrix)
- Whole genome 500 k SNP analysis
- Transcriptome analysis of human and mouse tissue
- Resequencing analysis

Transgenesis
- Generating transgenic mice and rats
- Knock out technology
- Inducible mouse models
- Behavioral studies

Molecular biology
- PCR
- dHPLC
- Pyrosequencing
- RT-PCR (LC480 Roche)

Microscopy
- Light and fluorescence microscope
- Confocal microscope (shared)

List of publications:


The Department of Neurodegeneration at the Hertie-Institute for Clinical Brain Research is devoted to unveil molecular mechanisms of movement disorders based on a combination of human genetics and functional validation in purified protein preparations, cell culture models, yeast, nematode mutants, and transgenic mice. A number of Parkinson’s disease genes have been identified by the Gasser lab, most recently PARK8 encoding leucin-rich repeat kinase-2 (LRRK2), a major and dominant cause of Parkinson’s disease. The small synaptic phosphoprotein α-synuclein (PARK1 and PARK4) is genetically and pathologically linked to Parkinson’s disease. The defining hallmark lesion of Parkinson’s disease, the Lewy body, is composed largely of synuclein fibrils. Moreover, Lewy bodies occur in several forms of dementia. In addition to these neuronal inclusions, α-synuclein fibrils are found in glial cytoplasmic inclusions of multiple system atrophy patients. The ubiquitin-ligase parkin (PARK2), mutations of which cause autosomal-recessive juvenile parkinsonism, may be functionally connected to α-synuclein. Two novel recessive genes link Parkinson’s disease to mitochondria and oxidative stress, namely the mitochondrial kinase PINK1 (PARK6) and the multifunctional protein DJ-1 (PARK7). The Kahle lab is investigating the structural defects of Parkinson’s disease relevant proteins, study the cell biological consequences of these protein aberrations, and develop animal models of Parkinson’s and related disorders.

Collectively, we currently focus on the following projects:

**PARK1 / α-synuclein**
- Cell culture models of α-synucleinopathy
  - Study of cellular consequences of post-translational modification (phosphorylation, ubiquitination)
- Nemotode models of α-synucleinopathy
  - Genetic networks influencing α-synuclein toxicity
- Transgenic mouse models of α-synucleinopathy
  - Synaptic transport
  - Solubility
  - Neuropathology
  - Deterioration of cognitive and locomotor functions
- Cross-breeding with additional risk genes

**PARK2 / Parkin**
- Regulation of parkin ubiquitin ligase activity by phosphorylation
- Involvement in unfolded protein stress
- Yeast two-hybrid screen for interactors

**PARK3 / unknown**
- Fine mapping of the locus
PARK6 / PINK1
Involvement in oxidative stress response

PARK7 / DJ-1
Structure / function analysis
+ Cell culture assays of anti-oxidative activity
+ Biophysical determination of solution structure

Neuropathology

PARK8 / LRRK2
Identification of pathological signal transduction pathways

Selected publications:

The Molecular Genetics Laboratory (MGL) at the University Eye Hospital Tuebingen has been established in 1992. It is one of very few medical genetic research labs embedded in an ophthalmologic clinic in Germany. The lab maintains a patients sample registry which includes more than 15,000 samples of patients with ocular disorders and their family members.

One main focus of our research program is color vision and the hereditary color vision deficiencies. We have performed studies on the structure of the red-/green-opsin gene cluster, with respect to red-/green-colorblindness and blue cone monochromacy. To be mentioned are also the publications of the identification, cloning and characterisation of the causative genes for autosomal recessive achromatopsia (rod monochromacy, total colorblindness).

Another main research project focuses on the causes of hereditary optic neuropathies. In the past we have worked intensively on the maternally/mitochondrially inherited Lebers hereditary Optic Neuropathy /LHON. Recently we are working on the autosomal dominantly inherited Optic Atrophy Type Kjer (ADOA). Our lab was substantially involved in the identification, cloning and characterisation of the first causative gene.

Our most recent project deals with the identification of hereditary factors in glaucoma.

The lab has profound expertise in nucleic acids purification and analysis, including DNA and RNA isolation, DNA and cDNA cloning, pulsed field electrophoresis, Southern and Northern Blot analysis, PCR and RT-PCR, real-time PCR, marker genotyping, mutation detection (SSCP and Heteroduplex analysis) and DNA sequencing. Facilities for mammalian cell culturing are available and more recently, techniques for functional analysis of genes and gene products (e.g. cell transfections, SDS gel electrophoresis, Western blotting, immunocytochemistry, Ca^{2+}-Imaging) have been established.

Key publications:


of all cases with autosomal recessive achromatopsia. European Journal of Human Genetics 13:302-308.

Retinitis Pigmentosa (RP) refers to a group of blinding diseases affecting 1 in 4000 persons in the industrialized world, with symptoms at times noticeable already at an early age. Although progress is being made in elucidating their genetic etiology (mutations in about 40 genes have so far been identified to be associated with these diseases), very little is known about the exact mechanisms of degeneration and no treatment is yet available. In age-related macular degeneration (AMD), the cone-rich area of the retina is affected and one fears that this type of degeneration will become a more prevalent cause of blindness among the elderly than diabetic retinopathy and glaucoma combined.

As a result of a mutation or following injury, a number of critical alterations are induced in target neuronal cells. As the target cells degenerate, responses are, in addition, elicited in other cells (neuronal & non-neuronal) – responses that are likely to impact on the progression of the disease. Several mechanisms involving, e.g., glial cells under physiological conditions are activated also under pathological conditions. An increased endogenous production of various factors by glial cells is observed in response to neuronal stress and several studies have demonstrated the ability of many of these factors to promote neuronal survival, when applied exogenously. However, it is also well established that activation of non-neuronal cells leads directly and/or indirectly to increased expression of e.g., inflammatory cytokines, matrix metalloproteinases, and other molecules, many of which can contribute to accelerating the progress of the degeneration and/or to limiting the effectiveness of a treatment. In this context, it is particularly interesting to consider what occurs, for instance, in the case of RP, in which mutations are seldom found in cone-specific genes, but mostly in rod-specific genes. Yet, cone cells, which are essential for high acuity and daytime colour vision, are lost in the course of or following rod cell loss, suggesting indirect mechanisms.

The programme will examine the role of non-neuronal cells as modulators/mediators of degeneration of retinal cells. The studies involve examination of various models of retinal cell degeneration in which mutations in genes encoding for rod- and retinal epithelium-specific proteins lead to loss of photoreceptor cells. An understanding of the contribution of non-neuronal cells to secondary pathophysiological responses will allow us to control the activity of these cells, “tipping the scale” in favour of neuroprotection and regeneration – an important aspect when designing therapies.

Selected publications:


Epilepsy is one of the most devastating neurological diseases affecting about 1% of the population of the western countries. Despite certain progress in antiepileptic drug development in about 25% of cases epileptic seizures do not respond to conventional treatment therapies based on enhancement of inhibitory GABAergic synaptic processes in the brain. Recently neuropeptides and neurotrophic factors emerged as strong regulators of excitatory and inhibitory synaptic transmission in the CNS thus offering a potent tool to counteract the seizure activity. However, it is not clear what the role of these agents is in mechanisms of epileptogenesis.

We explore the role of neuropeptides and neurotrophic factors in modulation of excitatory and inhibitory synaptic transmission and epileptogenesis in the brain. We use respective knock-out and overexpressing mice in combination with in vivo epilepsy models (kindling) and in vitro approaches (patch-clamp in brain slices). One of the specific aims of this research line is to use both direct and indirect targeted gene transfer of neuropeptides and neurotrophic factors into the different brain regions to investigate mechanistic aspects of their action on synaptic transmission and epileptogenesis.

**Histology**
- Immunocytochemistry
- Histochemistry
- Other common histological stainings

**Molecular biology**
- *In situ* hybridization
- PCR
- ELISA

**Cellular neurophysiology**
- Patch-clamp in brain slices using 'blind approach'
- Patch-clamp in brain slices using infrared video visualization of cells
- Patch-clamp in brain slices of GFP-labeled transplanted and endogenous stem cells
- Field recordings in brain slices

**Models of epilepsy**
- Conventional kindling
- Rapid kindling
- Status epilepticus

**Cell culture**
- Primary cell culture
- Neurospheres
- Brain slices

**Microscopy**
- Light and fluorescence microscope
- Stereology equipment
• Confocal microscope (shared)

Selected publications:


PROJECT 9

Role of endoplasmic reticulum (ER) and unfolded protein response (EPR) in in vivo and in vitro models of neurodegeneration (Responsible Supervisor: Prof. WIELOCH, Lund, Sweden)

The brain needs a constant supply of oxygen and glucose in order to generate energy for maintenance of brain function. During cerebral ischemia, when blood flow to the brain is severely hampered by occlusion of brain major arteries, by for example a blood clot (stroke), or due to cardiac arrest, energy production by brain cells rapidly ceases and detrimental processes are activated. Upon reperfusion by for example clot lysis or resumption of cardiac function, energy formation is transiently resumed by neurons. If ischemia is sufficiently dense and prolonged, neurons succumb within hours or days. Several mechanisms have been implicated in this process, including glutamate and calcium toxicity, free radical damage, inflammation, apoptosis and programmed cell death. In brain tissue that survives the ischemic insult, adaptive responses are activated leading to rewiring of neuronal networks and remodelling of synapses.

We have established in vivo and in vitro rodent models of cerebral ischemia, trauma and oxygen glucose deprivation, respectively. Using these models we focus our research on mechanisms of cell death involving mitochondria and the endoplasmic reticulum, as well as postischemic brain plasticity.

Work and methods description Dr. Toresson

Dr. Toresson recently received funding from the Swedish Research Council to establish his research group within Prof. Wieloch’s division. The work is focussed on structural plasticity and its functional implications for physiology and pathology. Within NEUROTRAIN, projects are centred on stress-induced structural dynamics of the endoplasmic reticulum (ER) and how these processes relate to known ER stress pathways. It is known that an ischemic insult leads to cessation of protein synthesis and that at least part of the unfolded protein response is triggered within the ER but how this contributes to protection, cellular damage or death is unknown.

We are using primary and organotypic cultures of the hippocampus to study these dynamic processes by live cell imaging with confocal microscopy. Neurons are transfected with various fluorescent protein constructs and transgenic mice are generated to enable imaging of slices and, in the future, the brain in vivo.

Key references:


Molecular studies in protein folding and pathological mechanisms of mitochondrial dysfunction (Responsibile Supervisor: Dr. UEFFING, Munich, Germany)

Protein misfolding and aggregation are among the central pathological hallmarks of neurodegenerative disorders, including not only prevalent diseases such as Alzheimer’s and Parkinson’s disease, but also such rare diseases as Huntington’s disease and retinal dystrophies where no therapeutic strategy has been so far developed. We have investigated the role of Cdc48p, the highly conserved yeast orthologue of Valosin-containing protein (VCP/p97). VCP is an effector protein for polyglutamine diseases and when mutated, is an inductor for the progressive dominant human disorder “inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia” (IBMPFD). Expression of VCP mutants with impaired function result in accumulation and aggregation of membrane-associated polyubiquitinated proteins and ER stress. From our recent work on mutant cdc48, we propose a direct mechanism of interaction between protein aggregation, ER stress and functional impairment of mitochondria. We aim to investigate the function of normal human VCP versus mutant VCP with respect to pathological mechanism of mitochondrial dysfunction that we have observed with mutant Cdc48p/VCP causing ER stress (Braun et al. submitted, Zischka et al. submitted). Since identification of molecular mechanisms underlying degeneration and cell death associated with Cdc48p/VCP bears implications for a variety of diseases characterized by abnormal protein deposits, Cdc48p/VCP-mediated cell death may therefore serve as a model for programmed cell death in human degenerative disorders. The analysis and subsequent identification of the systemic consequences of misfolded protein aggregation and their pathophysiological consequences within a cell can contribute to understanding mechanisms of neurodegenerative diseases and potentially lead to novel therapeutic approaches.

The training in this subproject will involve high-end bioanalytical and proteomic methodology including nanoLiquid-Chromatography (nLC) separation of proteins and mass-spectrometry by MALDI-TOF, Q-TOF and MALDI-MS/MS. Functional analysis of normal and mutant protein variants will include survival, stress and apoptosis assays on primary neuronal and glial cell lines, permanent cell lines, analysis of protein-protein interaction, protein modification and aggregation, immunohistochemistry and biochemical assays analysing protein turnover and mitochondrial function, recombinant expression, primary cell culture, cDNA-arrays, bio-computing and animal facilities (German Mouse Clinic) at the GSF.

The GSF Campus with more than 20 different institutes and departments offers a multifaceted research biotope. The Lab of Dr. Ueffing runs the central Proteome Core Facility within the GSF Gene Analysis Center (GAC), an integrated facility for proteomics, gene arrays and DNA analysis. Dr. Ueffing coordinates the German Federal Government funded National Research Cluster ‘Analysis of Membrane-coupled Protein Complexes’ that focuses on developing proteomic methodology for the analysis of membrane proteins and membranous organelles and partners "INTERACTION PROTEOME" Europe's largest contracted EU research network for proteomics. This offers a variety of training opportunities embedded into an internationally structured
research network. Training extends from human genetics, functional genomics and proteomics to advanced cell biology.

Selected publications:
